

This document is an addendum to
the ANKOM^{TDF} Dietary Fiber Analyzer Operator's Manual.

This document does NOT replace the Operator's Manual.



Rev 09/30/22

This page intentionally left blank

Table of Contents

Introduction	5
Accessories.....	5
Instrument Configuration	6
Dietary Fiber Analysis Support Items.....	10
Blanks	10
Method Comparison (Manual vs. Automated).....	11
IDF/SDF Analysis (AOAC 2011.25).....	12
TDF Analysis (AOAC 2009.01)	29
Status Screen	45
Fault Handling.....	45
Diagnostics Mode	45
Appendix A – Reagents (AOAC 2009.01/2011.25).....	47
Appendix B - Analytical Procedures.....	49
Appendix C- TRIZMA Line Flush	55

This page intentionally left blank

Introduction






The ANKOM^{TDF} Dietary Fiber Analyzer was designed to automate multiple gravimetric methods. This document describes how to configure and use the ANKOM^{TDF} Dietary Fiber Analyzer for the methods defined by AOAC 2009.01/2011.25 & AACC 32-45.01/32-50.01.

NOTE:

To conserve space, the instrument's Touch Screen Display only refers to the AOAC methods. Therefore, this document will primarily refer to the AOAC methods. Please review this entire document and the ANKOM^{TDF} Dietary Fiber Analyzer Operator's Manual before you begin using the instrument for these procedures.

Accessories

The following accessories are needed to run the identified Dietary Fiber methods on your ANKOM^{TDF} Dietary Fiber Analyzer, and should have come with your instrument or with your upgrade kit.

1 – Amylase/AMG Container (TDF57)	1 – Acetic Acid Container (TDF59) & Fluid Tube Label	1 – Sodium Maleate Buffer Container (TDF60) & Fluid Tube Label	6 – Glass Filtrate Cups (TDF97)
1 – Trizma Base Container (TDF58)			
			
			

Instrument Configuration

To run the identified Dietary Fiber methods, your ANKOM^{TDF} Dietary Fiber Analyzer must be configured with the proper:

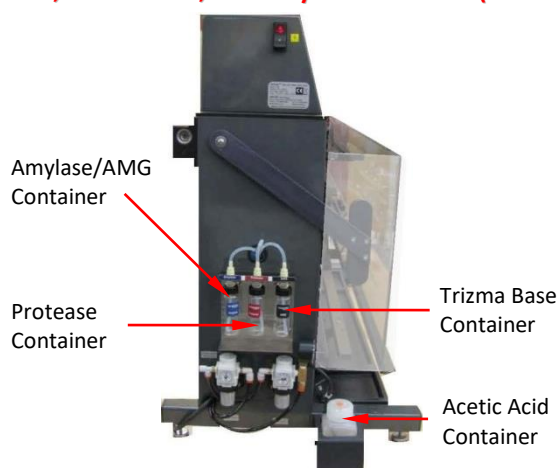
- Chemical and Enzyme Containers and Glass Filtrate Cups
- Fluid Tube Labels
- Registration Code
- Mixer Duty Cycle

To configure your instrument to run the identified Dietary Fiber methods, follow the steps below.

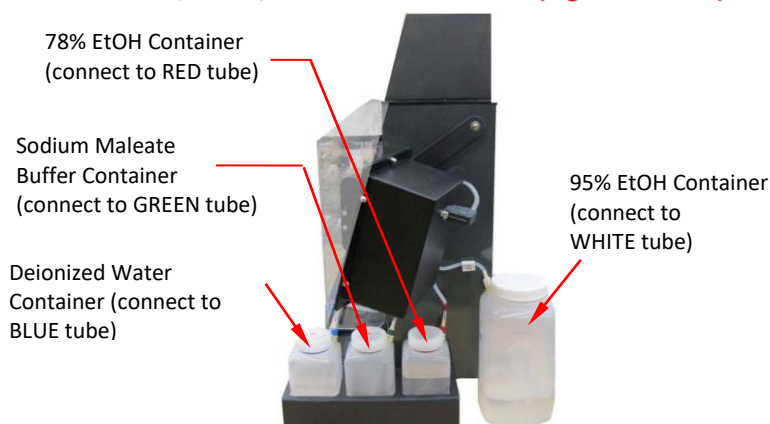
1. Install chemical and enzyme containers and place glass filtrate cups at each station.

If you have used the instrument for the AOAC 991.43/985.29/2001.03 methods, the AOAC 2009.01/2011.25 configuration will require you to replace some of the chemical and enzyme bottles. Configure the chemical and enzyme bottles according to the pictures below (continued on the next page).

Acetic Acid, Trizma Base, and Enzyme Containers (Left Side View)



Buffer, Water, and Ethanol Containers (Right Side View)



Place glass filtrate cups at each of the six stations for collection of low molecular weight dietary fiber filtrate for HPLC analysis.

2. Install tube labels for this method.

Color-coded labels are attached to each fluid tube to help ensure that the correct chemicals are connected to the correct tubes. For the AOAC 2009.01/2011.25 configuration, the additional labels should be installed as follows.

- 2.1 Apply the **BLACK** Acetic Acid Tube label to the HCl tube on the left side of the instrument.
- 2.2 Apply the **GREEN** Sodium Maleate Buffer label to the Buffer tube on the right of the instrument.

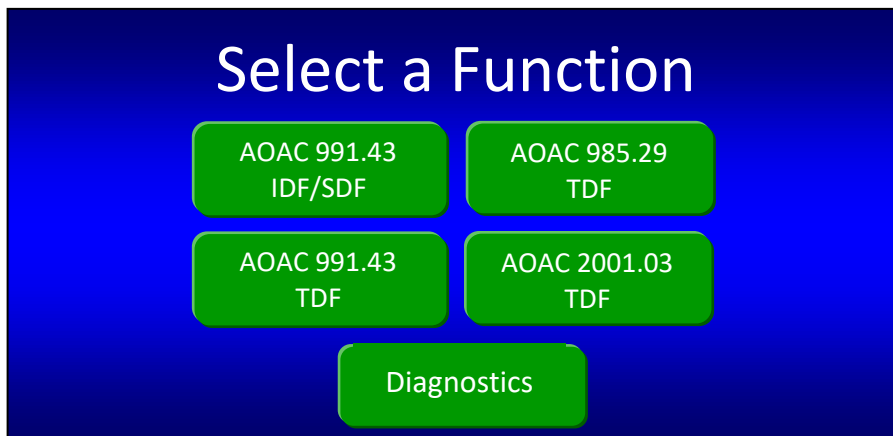
NOTE:

If you purchased your instrument with the AOAC 2009.01/2011.25 methods already enabled, you will see on the Touch Screen Display the buttons for those procedures on the "Select a Function" screen. In that case, you do NOT need to do steps 3 and 4 below because they were already done at the factory.

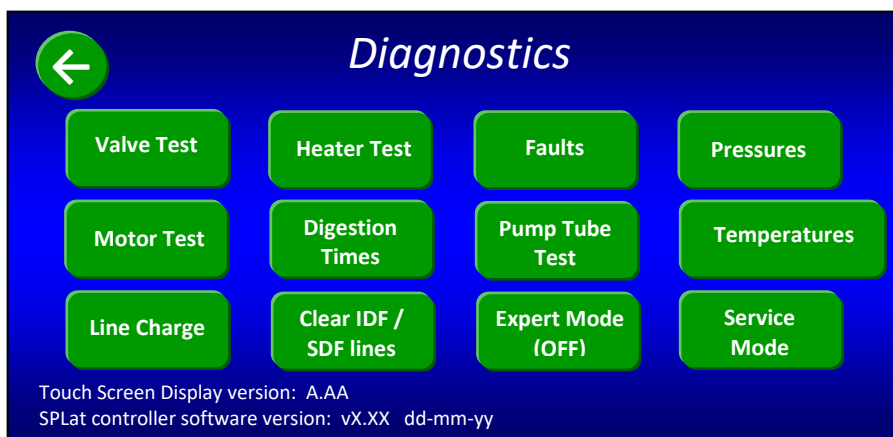
3. Enter the Registration Code into the instrument to enable the functionality.

If you purchased your instrument without the AOAC 2009.01/2011.25 methods already enabled, and you are now implementing the upgrade kit, you must enter the Registration Code by following the steps below.

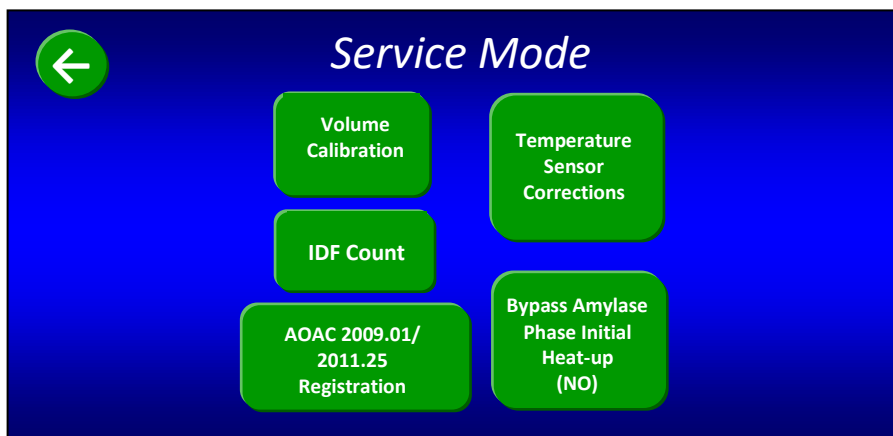
- 3.1 Turn the instrument ON. After the instrument initializes, you will see the "Select a Function" screen on the Touch Screen Display.



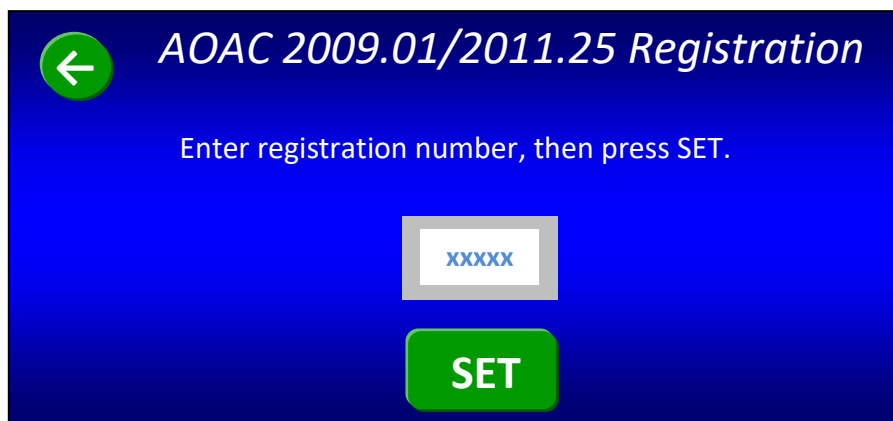
- 3.2 Press the **Diagnostics** button. The following screen will be displayed.



- 3.3 Press the **Service Mode** button. The following screen will be displayed.

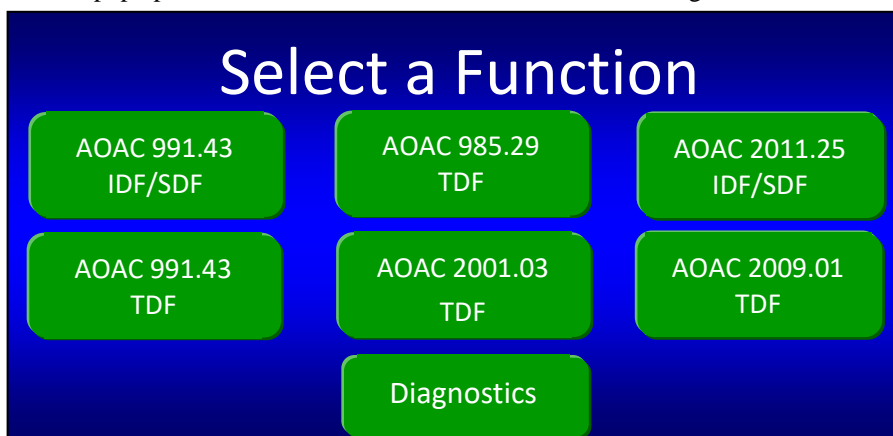


- 3.4 Press the **AOAC 2009.01/2011.25 Registration** button. The following screen will be displayed.



- 3.5 Press the white button with the gray border. Enter the 5-digit registration code provided by ANKOM and press the **SET** button. If you enter an incorrect number, the instrument will tell you to try again.

The unique code will unlock the additional programs. After properly setting the code, the message "AOAC 2009.01 / 2011.25 Enabled" will pop up on the screen for a short time. Then the following screen will be displayed.



As you can see, there are now two new buttons on the "Select a Function" screen that allow you to run the AOAC 2009.01/2011.25 methods.

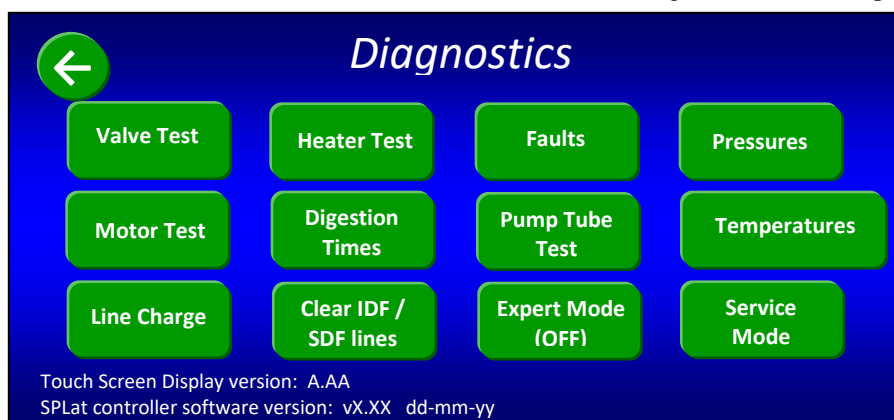
NOTE:

Although the instrument works in accordance with AACC 32-45.01/32-50.01, the Touch Screen Display only refers to the AOAC methods.

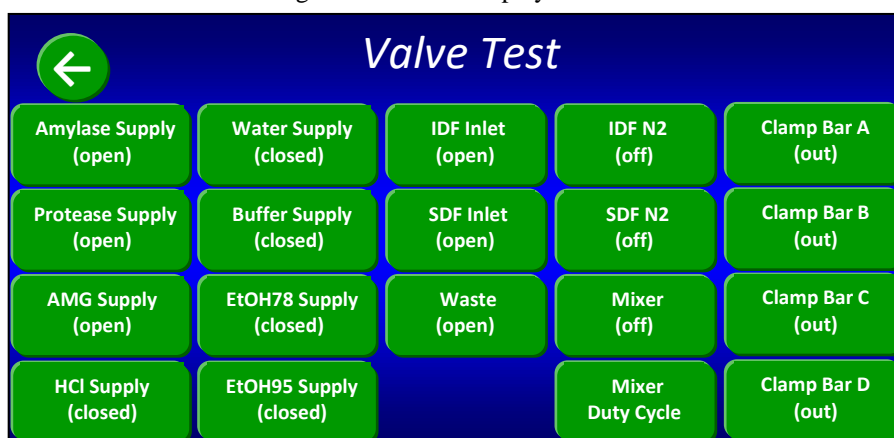
4. Verify that the Mixer Duty Cycle is set appropriately.

The mixer duty cycle for the AOAC 2009.01/2011.25 methods is "5". To verify the setting, follow the steps below.

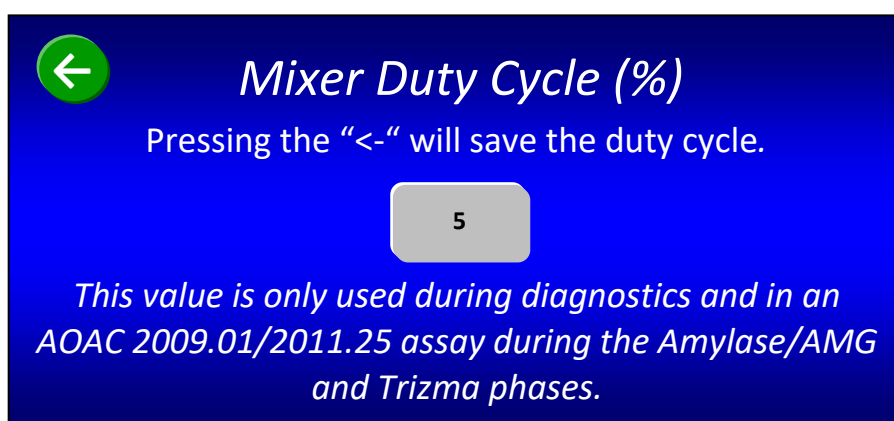
4.1 Press the "Diagnostics" button on the "Select a Function" screen. The following screen will be displayed.




4.2 Press the "Valve Test" button. The following screen will be displayed.



4.3 Press the "Mixer Duty Cycle" button. The following screen will be displayed.



4.4 If the number on the gray button is NOT "5", press the gray button and enter "5".

4.5 Press the back  button to save the duty cycle number. This will configure the mixer to run 5% of the time during the mixing process to comply with AOAC 2009.01/2011.25 specifications.

4.6 Press the back  button two more times to return to the "Select a Function" screen.

Your instrument is now configured to run the AOAC 2009.01/20011.25 methods.

Dietary Fiber Analysis Support Items

The following support items needed to perform the fiber analysis may be purchased separately:

Item	ANKOM Part #
Heat Sealer for sealing the filter bags	HS (120V), HSi (220V)
IDF Filter Bags	DF-I
SDF Filter Bags (used for SDF and TDF procedures)	DF-S
IDF <i>Flow-Thru</i> Bags (TDF procedure only)	DF-FT
Solvent Resistant Marker	F06
Rinse Stand	TDF51
Diatomaceous Earth (DE)	DE1, DE2
Electronic Balance with four-place readout	
Electronic Balance Software	
Drying Oven	
Ashing Oven	
Protein Determination Equipment (Kjeldahl is recommended)	
Equipment to determine the Water: Alcohol Soluble dietary fiber (WASDF)	

Blanks

The following BLANK values (based on research done in the ANKOM lab) are the default values loaded in the IDF_SDF and TDF spreadsheets in the MS-Excel workbook provided with the instrument.

IDF (AOAC 2011.25)	-0.0052	SDF (AOAC 2011.25)	-0.0045	TDF (AOAC 2009.01)	-0.0034
---------------------------	----------------	---------------------------	----------------	---------------------------	----------------

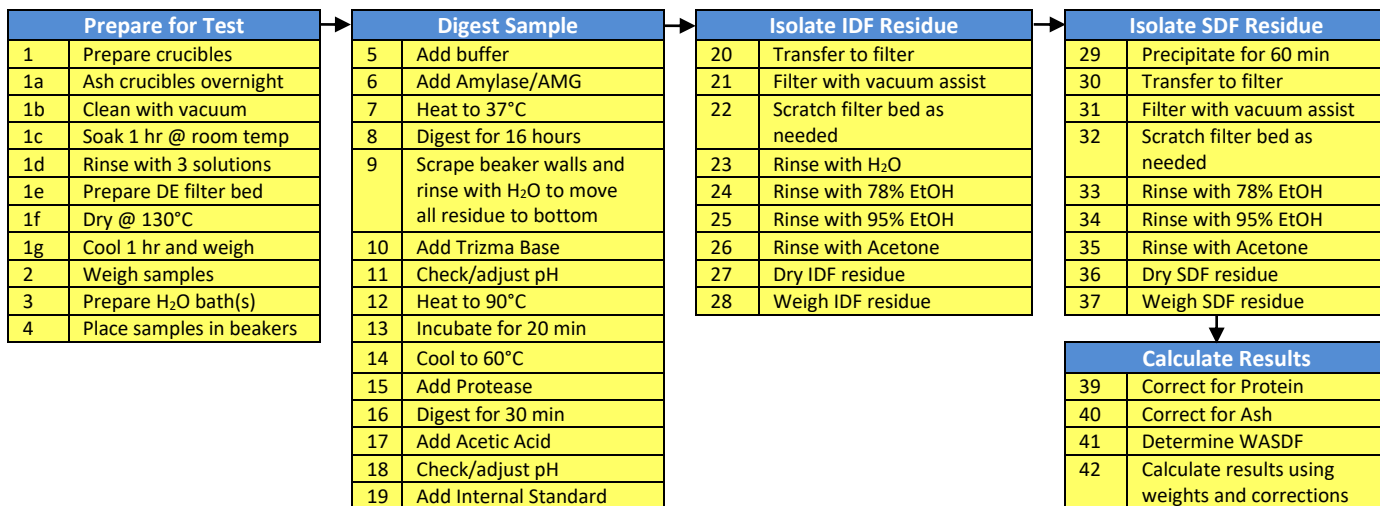
You do not need to run BLANKS with every set of samples. However, if you want to determine your own BLANK values, especially when you change chemical lots, **ANKOM recommends that the BLANK value in the IDF, SDF, and TDF calculations be the average of at least 18 BLANK values.**

If you determine your own BLANK values, you can overwrite the values currently in the MS-Excel spreadsheets.

Method Comparison (Manual vs. Automated)

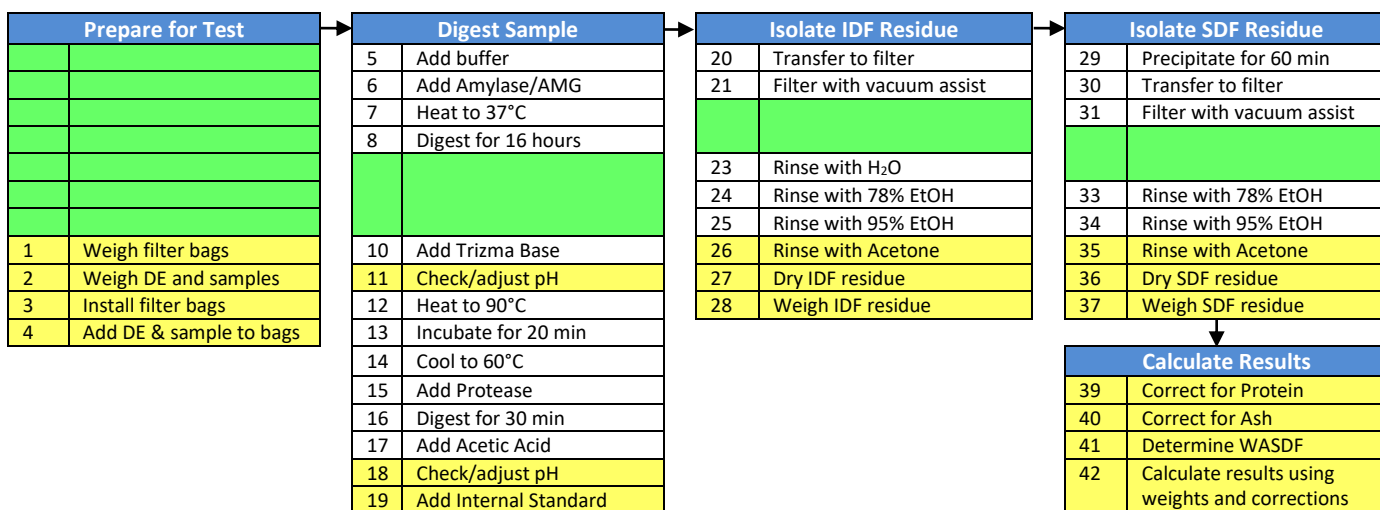
AOAC 2011.25 Method – IDF/SDF assay without automation

Below is the AOAC 2011.25 method flow done manually by a technician using glass crucibles and beakers, vacuum to assist with filtering, and water baths to control temperature and mixing. DE refers to Diatomaceous Earth.



AOAC 2011.25 Method – IDF/SDF assay with ANKOM^{TDF} Dietary Fiber Analyzer automation

Below is the AOAC 2011.25 method flow when using the ANKOM^{TDF} Dietary Fiber Analyzer. Steps highlighted in YELLOW represent tasks done by a technician. Steps highlighted in GREEN (1a-1g, 9, 22, and 32) are no longer needed because of Filter Bag Technology. All other steps (including mixing) are done automatically by the ANKOM^{TDF} Dietary Fiber Analyzer under computer control. DE refers to Diatomaceous Earth.



NOTE:

This section is just an overview of how the ANKOM^{TDF} Dietary Fiber Analyzer executes the AOAC 2011.25 method. Please see the “IDF/SDF Analysis (AOAC 2011.25)” and “TDF Analysis (AOAC 2009.01)” sections of this manual for detailed instructions on how to perform IDF/SDF and TDF assays with the analyzer.

IDF/SDF Analysis (AOAC 2011.25)

An IDF/SDF analysis measures the amount of IDF and SDF within a given sample. This requires the use of ANKOM DF-I and DF-S filter bags. When starting a new run, the ANKOM^{TDF} Dietary Fiber Analyzer must have bags installed at all stations (a total of twelve bags).

NOTE:

To enhance the productivity of your instrument, you can begin the IDF process for a new run while the SDF process of a previous run is finishing. See the “Productivity Enhancement” section of your ANKOM^{TDF} Dietary Fiber Analyzer Operator’s Manual for more details.

To perform an IDF/SDF analysis, follow the steps below.

1. Prepare chemicals and enzymes.

When using the ANKOM^{TDF} Dietary Fiber Analyzer for the AOAC 2011.25 methods, use the chemicals and enzymes referred to therein. See Appendix A of this manual for the list of chemicals and enzymes and the instructions for how to prepare them for use in this instrument.

2. For samples that have a fat content of 10% or higher, de-fat your samples as needed according to the official methods or your internal SOPs.

3. Label the filter bags using a Solvent Resistant Marker.



4. Prepare for data collection.

You will need a place to store the data collected during this analysis. For your convenience, a USB flash drive was included with the instrument and includes a Calculation Template that can be used for this analysis. Please read the “Instructions” tab in the MS-Excel file for information about the spreadsheets.

5. Weigh filter bags.

IMPORTANT: Using a Bag Weigh Holder is critical to eliminate the effects of static electricity during the weighing process.

Roll or fold each bag and place it in a tared Bag Weigh Holder. Place the Bag Weigh Holder in the center of a balance and record the weight.

**NOTE:**

Because different balances have different sensitivities, the Bag Weigh Holder should be placed in the center of the balance for best results.

6. Weigh Diatomaceous Earth (DE).

DE is used during fiber analysis to enhance the flocculation and filtration of the SDF fraction. Place ca 1g \pm 0.05g of DE in each of six tared and numbered tins and record the weights.

7. Weigh Samples.

Place 0.5 \pm 0.05 g of sample in each of six tared and numbered tins and record the weights.

NOTE:

A larger sample (1.0g) can be used to improve precision if necessary.

8. Confirm Nitrogen Supply and Pressure Ranges.

Make sure the Nitrogen supply in your lab is connected to the instrument and turned on. The High-Pressure Gauge on the left of the instrument should be set between 50-55 psi. The Low-Pressure Gauge on the right side of the instrument should be set at 4 psi. The accuracy of the Low-Pressure Gauge should be checked prior to each run by pressing the “Pressurize SDF” button on the control panel twice.

High Pressure Gauge
50-55 psi (3.5-3.8 bar)



Low Pressure Gauge
4 psi (0.3 bar)

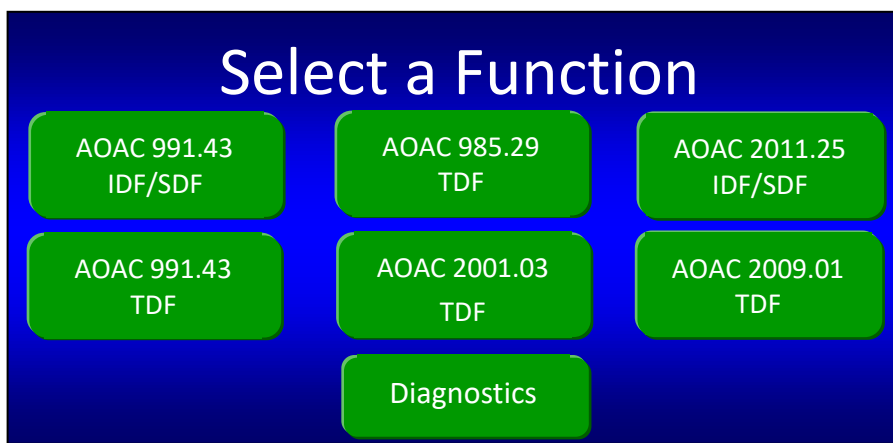
9. Turn the instrument power on.

When you turn the power on, the instrument will run through an initialization process and the Control Panel will turn on.

10. On the Touch Screen Display, select the instrument function you would like to perform.

The Control Panel on the ANKOM^{TDF} Dietary Fiber Analyzer uses Touch Screen technology. To operate the instrument, you will press identified buttons on the Touch Screen Display and buttons below the screen.

When your instrument is initialized and ready to operate, the following screen will be displayed.

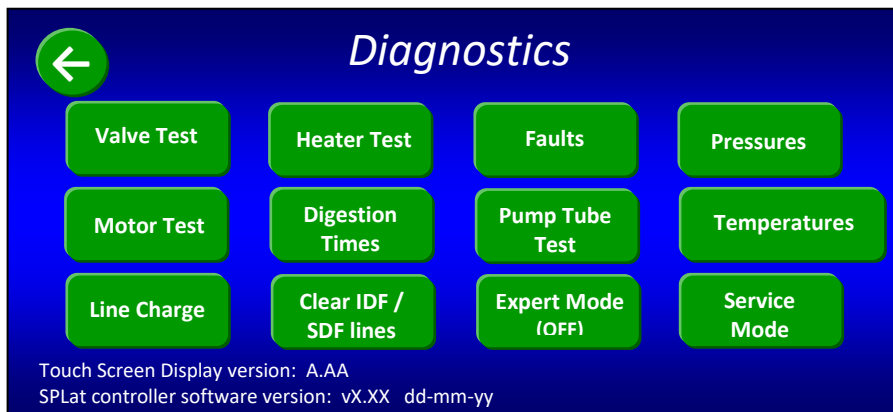


NOTE:

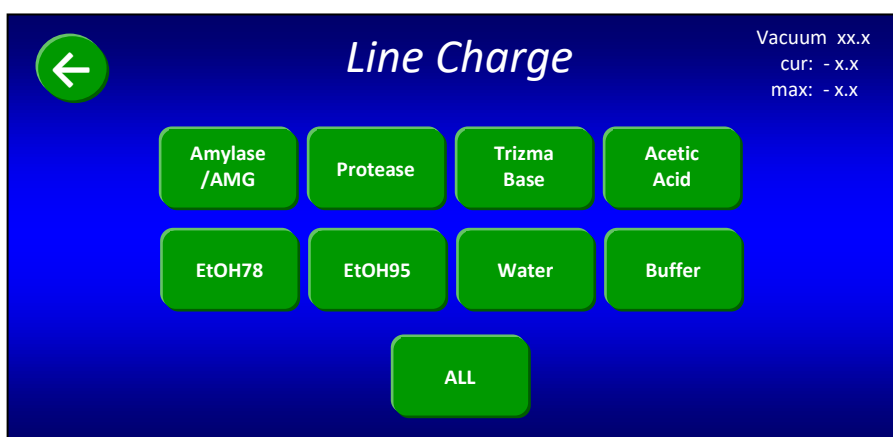
Although the instrument works in accordance with the AACC 32-50.01 method, the Touch Screen Display only refers to the AOAC methods.

- 10.1 If this is the first time the instrument is being operated after being installation, or if this is the first time the instrument is being operated after sitting unused for a period of time and was flushed according to Appendix C, the lines must be charged to avoid the faults: E13 (Tubing Failure) or E14 (Empty Chemical Container). To charge the lines follow the steps below.

10.1.1 Press the “Diagnostics” button on the “Select a Function” screen. The following screen will be displayed.

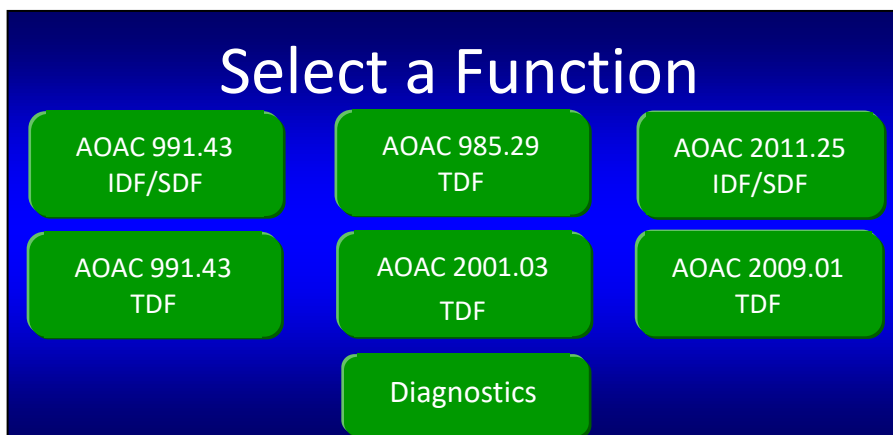


10.1.2 Press the “Line Charge” button. The following screen will be displayed.

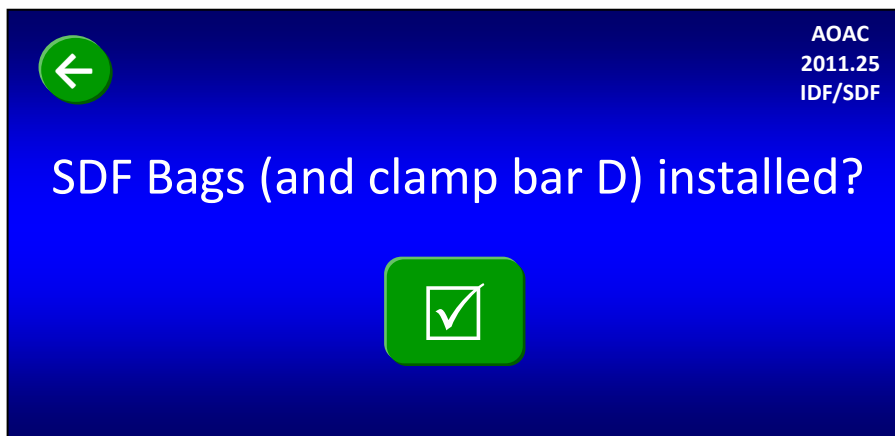


10.1.3 Press the “ALL” button to charge all of the lines. Each button will change color as the associated line is being charged. All lines are charged when all of the buttons return to their original green color.

10.1.4 Press the back button twice. The following “Select a Function” screen will be displayed again.



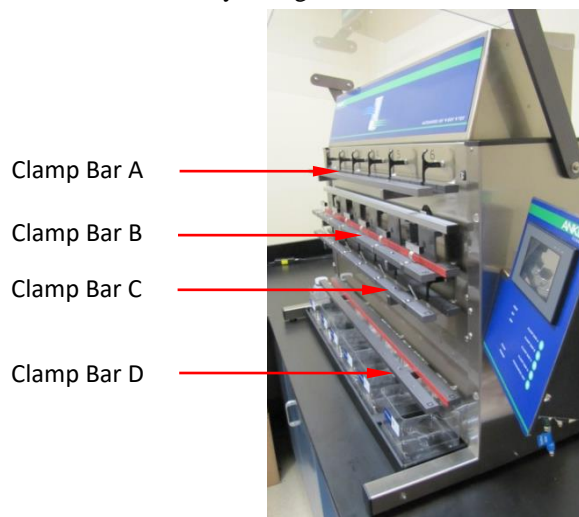
10.2 Press the “AOAC 2011.25 IDF/SDF” button. The following screen will be displayed.



Notice that the function you selected is now displayed in the top right corner of the screen and a back button is displayed in the top left corner of the screen.

11. Install SDF filter bags on the ANKOM^{TDF} Dietary Fiber Analyzer.

11.1 Remove Clamp Bars A, B, C, and D by lifting them off of the locator rods.



11.2 Gently pull the black SDF Delivery Nozzle out toward you.



- 11.3 Place a labeled and weighed SDF bag up underneath the SDF Delivery Nozzle so that the Delivery Nozzle is inside the top part of the bag. Pull the bag up so that the top of the bag is about 35 mm (1.375 inches) above the top of Clamp Bar C and return the Delivery Nozzle to its original position. This will hold the back of the bag in place.

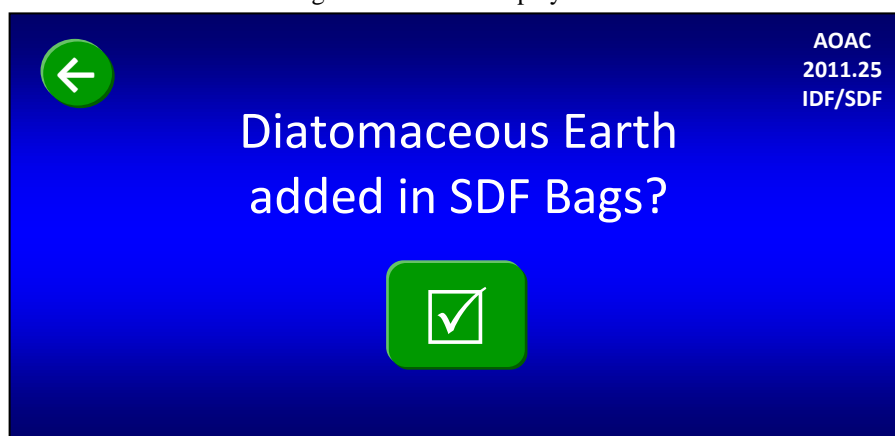
**IMPORTANT:**

As part of normal operation, solution from the IDF bag will flow into the SDF bag. Therefore, when installing the SDF filter bags it is very important to position them high enough vertically so that at least 20 mm (0.75 inches) of the filter part of the IDF bag can fit inside the top of the SDF bag.

- 11.4 With the bag being held by the Delivery Nozzle, center it horizontally between the lines on the back part of Clamp Bar C.



- 11.5 Repeat the previous steps for all six stations.
- 11.6 Re-install Clamp Bar D by setting it on the locator rods. Make sure the letter is on the top of the bar and the rubber material is facing in toward the instrument.
- 11.7 Flatten the bags to remove any wrinkles.
- 11.8 With fingers away from the clamp bars, press the check mark (☑) button on the Touch Screen Display to pinch the bags just above the filter. The following screen will be displayed.



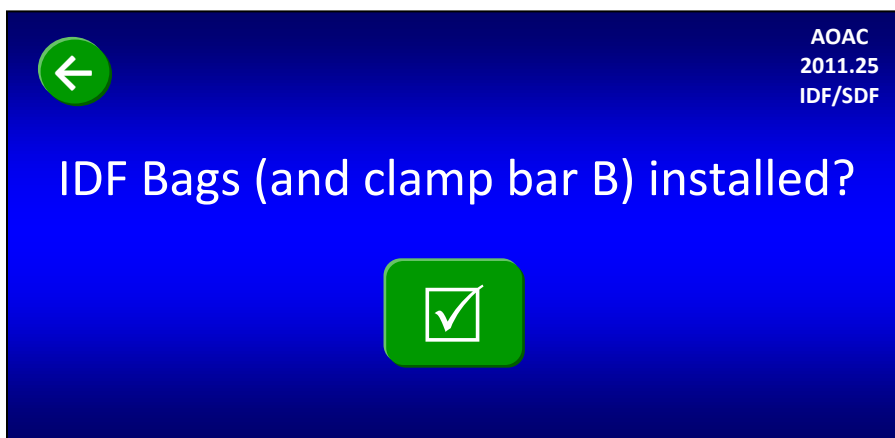
12. Add DE to the SDF filter bags on the ANKOM^{TDF} Dietary Fiber Analyzer.

- 12.1 Open the top of the SDF bag and add a weigh tin of DE to the bag by folding the tin and then dipping it down into the bag below the tip of the Delivery Nozzle.



IMPORTANT: When adding DE to the filter bags it is very important to keep it below the tip of the Delivery Nozzle so that the DE material can be properly rinsed.

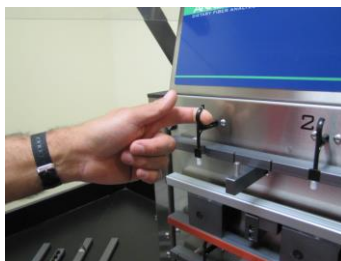
- 12.2 If necessary, rinse the weigh tin/weigh boat with 78% EtOH to ensure complete transfer.
12.3 Repeat the previous steps for all six stations.
12.4 Press the check mark (✓) button on the Touch Screen Display. The following screen will be displayed.



NOTE: After you confirm that the DE is added, the Clamp Bar D button on the Control Panel is disabled until the SDF process completes.

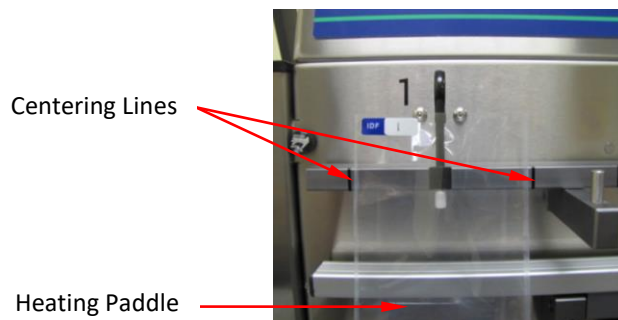
13. Install IDF filter bags on the ANKOM^{TDF} Dietary Fiber Analyzer.

- 13.1 Gently pull the black IDF Delivery Nozzle out toward you.



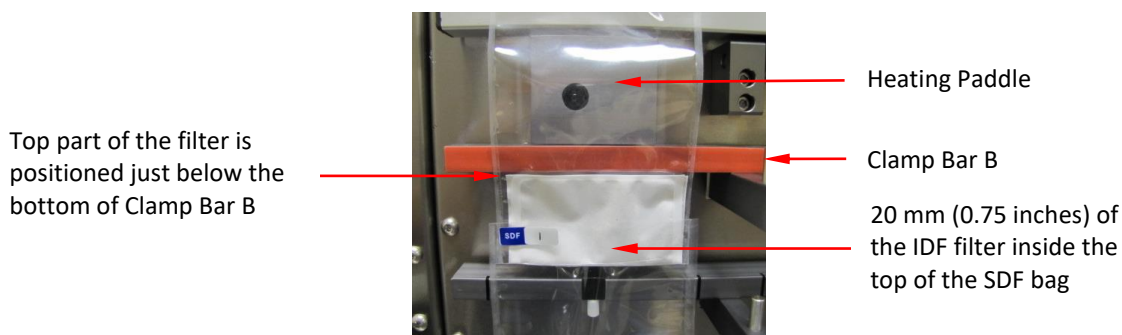
- 13.2 Place a labeled and weighed IDF bag up underneath the Delivery Nozzle so that the Delivery Nozzle is inside the top part of the bag. Pull the bag up so that the top of the filter part of the IDF bag is just below the bottom of Clamp Bar B and return the Delivery Nozzle to its original position. This will hold the back of the bag in place.

- 13.3 With the bag held by the Delivery Nozzle, center it horizontally between the Centering Lines on the back part of Clamp Bar A.

**IMPORTANT:**

For proper mixing during the IDF process the IDF bags must be horizontally centered over the Heating Paddles and between the Centering Lines on the back part of Clamp Bar A.

- 13.4 Place at least 20 mm (0.75 inches) of the filter section of the IDF bag inside the top of the SDF bag to allow for the flow of liquid into the SDF bag after the IDF process is complete.



- 13.5 Repeat the previous steps for all six stations.
- 13.6 Re-install Clamp Bar B by setting it on the locator rods. Make sure the letter is on the top of the bar and the rubber material is facing in toward the instrument.
- 13.7 Flatten the IDF bags to remove any wrinkles.
- 13.8 With fingers away from the clamp bars, press the check mark (☑) button on the Touch Screen Display to pinch the IDF bags just above the filter. The mixing pads will make contact with the bags when Clamp Bar B pinches the IDF bags. The following screen will be displayed.



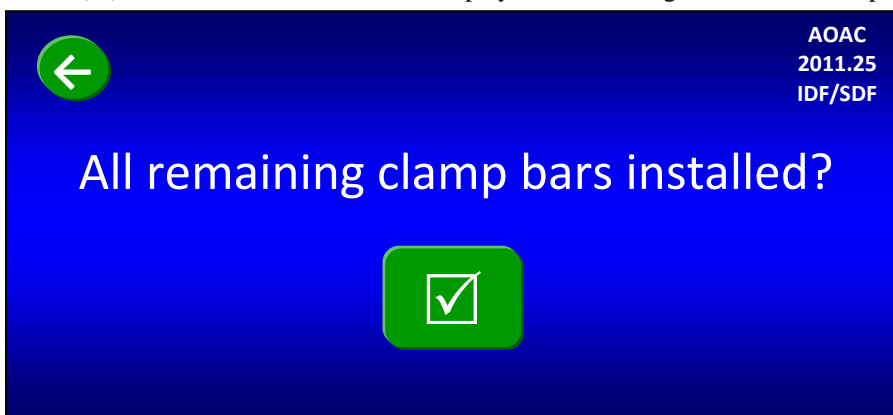
14. Add samples to the IDF bags.

- 14.1 Open the top of the IDF bag and transfer the sample from a weigh tin into the IDF bag by folding the tin and then dipping it down into the bag below the tip of the Delivery Nozzle.

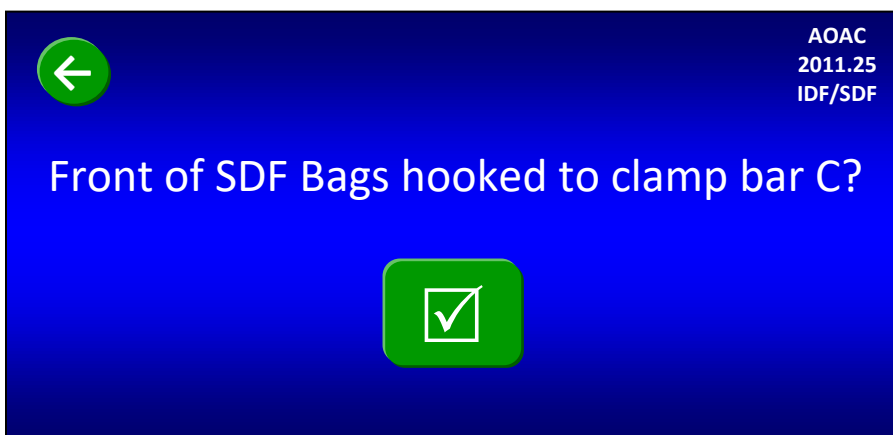


IMPORTANT: When adding sample to the filter bags it is very important to keep it below the tip of the Delivery Nozzle so that it can be properly rinsed.

- 14.2 Rinse the weigh tin/weigh boat with as little Deionized (DI) or Distilled (DW) water as possible (maximum 3 ml) to ensure complete transfer.
- 14.3 Repeat the previous steps for all IDF stations.
- 14.4 Re-install Clamp Bar A by setting it on the locator rods. Make sure the letter is on the top of the bar and the rubber material is facing in toward the instrument.
- 14.5 Press the check mark (☑) button on the Touch Screen Display. The following screen will be displayed.



- 14.6 Make sure that all clamp bars are installed with the letter on the top of the bar and the rubber material facing in toward the instrument. Press the check mark (☑) button on the Touch Screen Display. The following screen will be displayed.



15. Hook the front of each SDF bag in place.

- 15.1 Secure the SDF filter bags in place with the hooks located on the front part of Clamp Bar C by pulling up the middle section of the front of the filter bag evenly and tightly, lean the bag against the hook as the bag is lowered, allowing the bag to catch onto the hook. There should be at least a 1/4" of the top of the bag sitting above the hook.

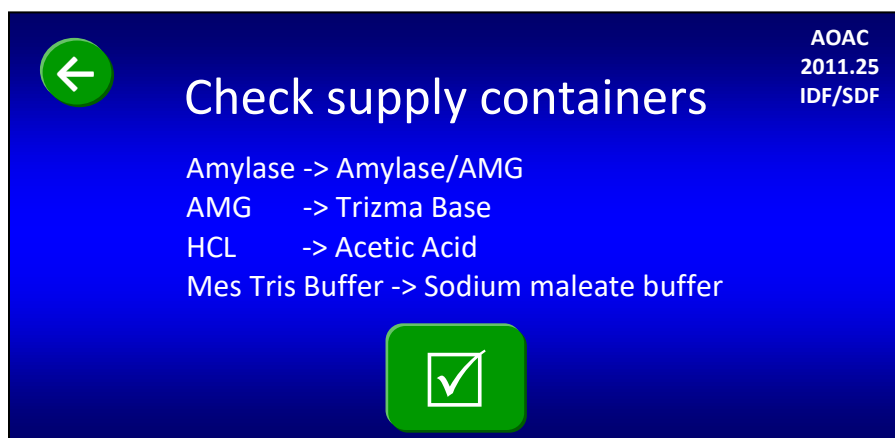


Hook on front part of
Clamp Bar C

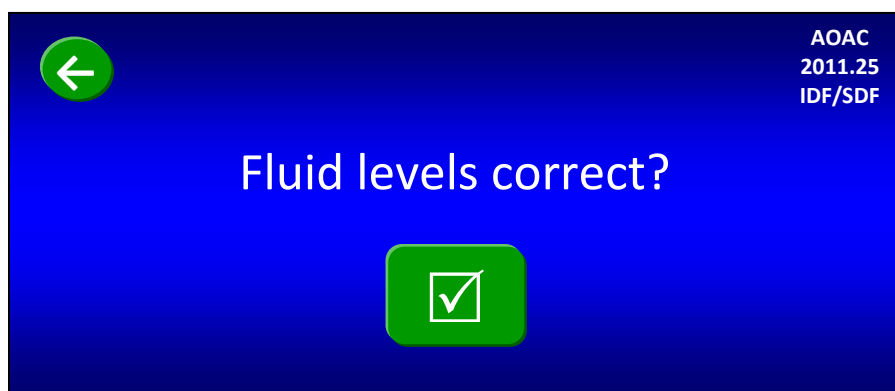
NOTE:

Hooking the bags in place will put a physical hole in the front of each SDF bag.

- 15.2 Press the check mark (☑) button on the Touch Screen Display. If you ran a method other than AOAC 2011.25 or 2009.01 prior to this run, the following screen will be displayed.

**16. Verify that the fluid containers are correct for this procedure.**

Verify that the fluid supply containers are configured according to the screen above and press the check mark (☑) button. The following screen will be displayed.



17. Fill fluid containers.

To ensure that you have enough fluids to run a complete IDF/SDF procedure, you must begin with at least 30 ml of the Amylase/AMG mixture, 15 ml of Protease, 30 ml of Trizma Base, and fluid levels above the Minimum Level lines on the remaining chemical containers. Add fluids and enzymes as necessary.

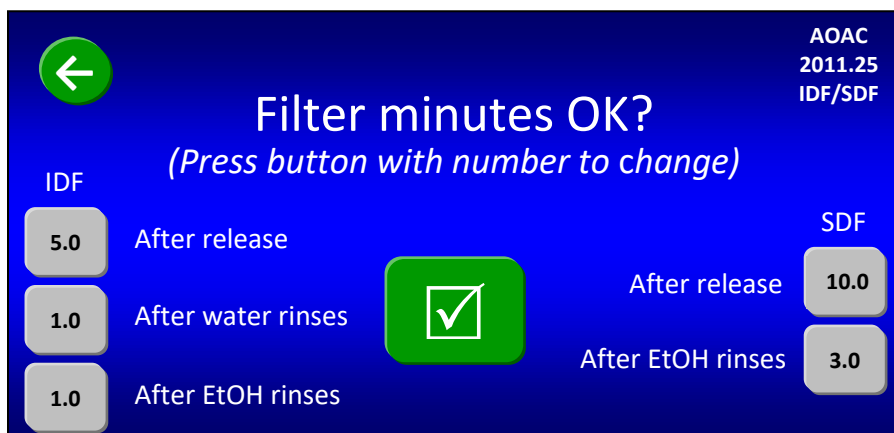
With all fluid containers filled to the proper levels, press the check mark (☑) button on the Touch Screen Display. The following screen will be displayed.



18. Confirm Nitrogen Supply Settings.

Make sure the Nitrogen supply in your lab is connected to the instrument and turned on. The pressure gauges on the instrument should show 50-55 psi on the left and about 4 psi on the right.

Press the check mark (☑) button on the Touch Screen Display. The following screen will be displayed.



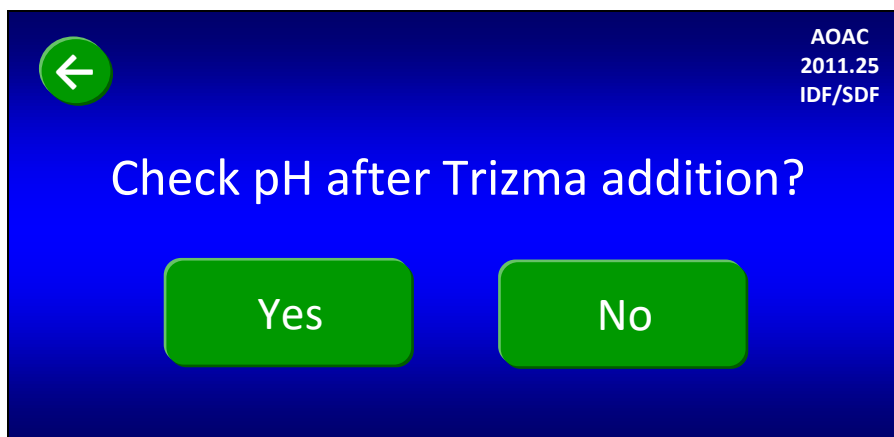
19. Set filter times (in minutes).

Because different samples take different amounts of time to filter, the above screen allows you to set your filter times. To change any of the times shown on the screen, press the specific gray button. A number pad will be displayed that will allow you to enter the time that you want. The times you enter will remain until you change them again.

NOTE:

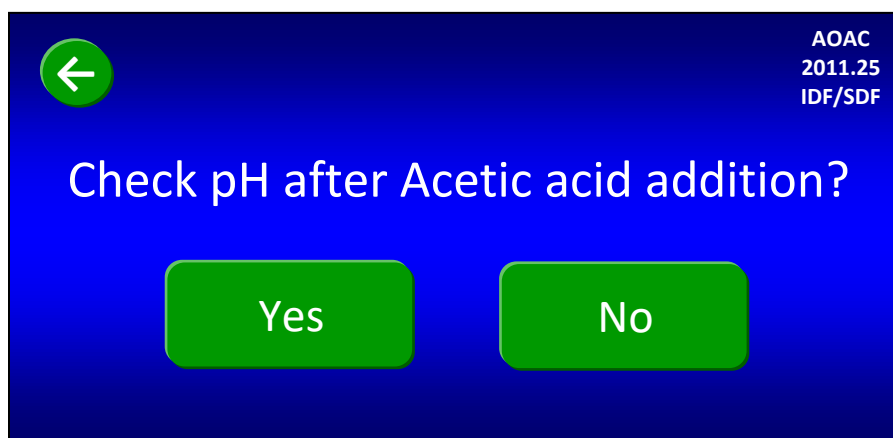
The initial filter times shown when you first run the instrument are based on factory experience. During filtration the computer allows you to bypass the filter time if you notice the filtering is complete. The computer also allows you to add time during filtering if needed (see the "Status Screen" section of the Operator's Manual for more detail).

When all of the times shown on the screen are what you want, press the check mark (✓) button on the Touch Screen Display. The following screen will be displayed.

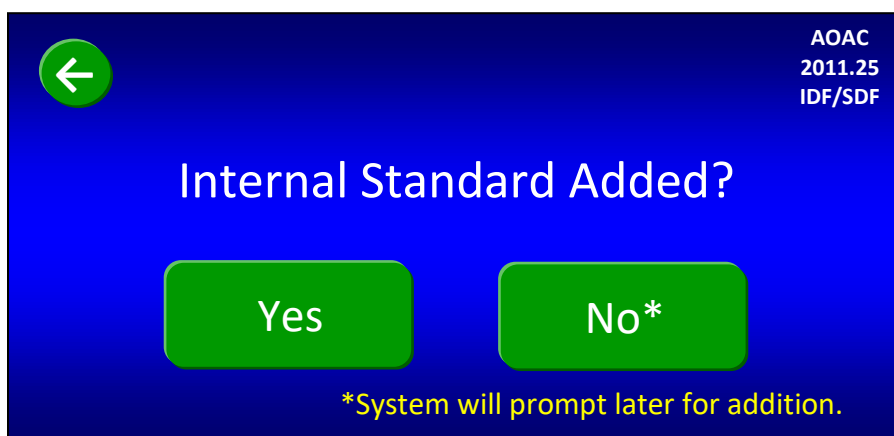


20. Set the Manual pH checks.

If you plan to check the pH after the required Trizma base is added (during the IDF process), press the “Yes” button on the screen above. Otherwise, press the “No” button. The following screen will be displayed.

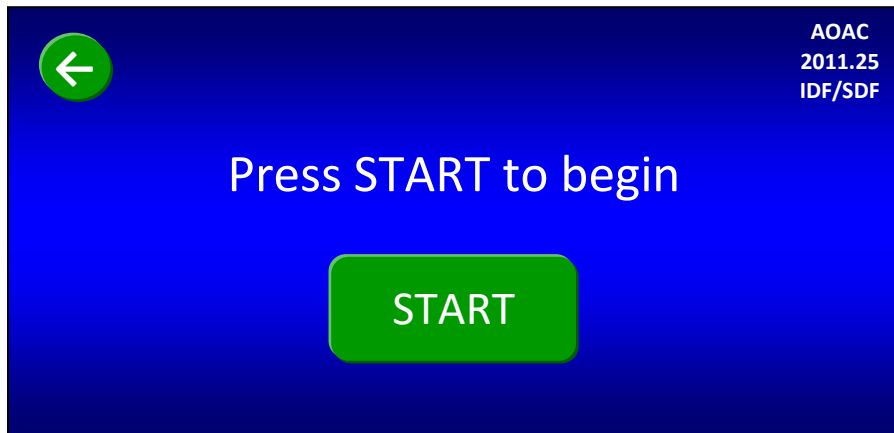


If you plan to check the pH after the required Acetic acid is added (during the IDF process), press the “Yes” button on the screen above. Otherwise, press the “No” button. The following screen will be displayed.



21. Add Internal Standard now or tell the instrument to prompt you later.

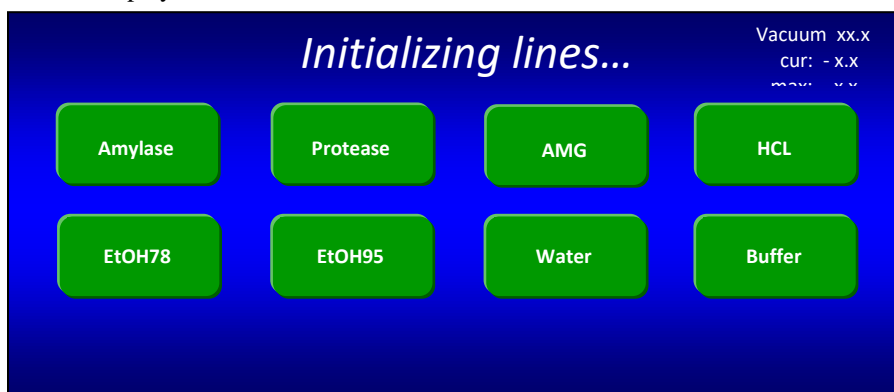
You can either add 1 ml of the Internal Standard (as per the AOAC 2011.25 method) before starting the instrument, or you can be prompted to add it after the pH checks. If you want to add it before starting the instrument, add it now to each IDF bag and press the “Yes” button. If you want to add it after the pH checks (during the IDF process), press the “No” button on the screen above and the instrument will prompt for the addition later. The following screen will be displayed.



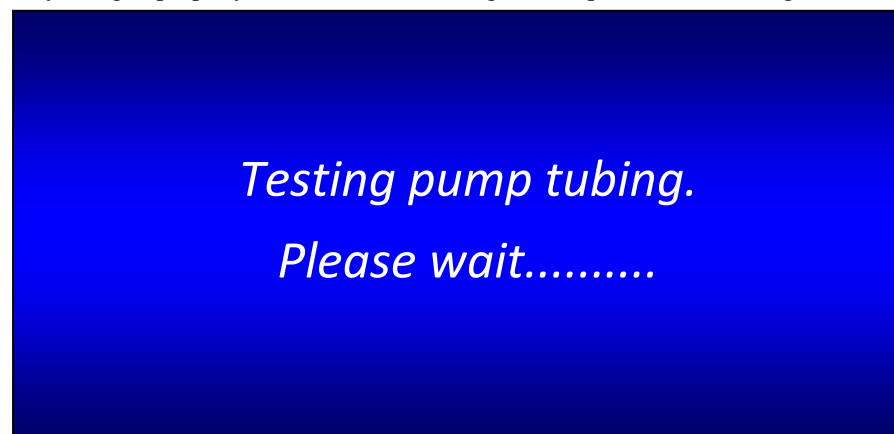
22. Start the instrument.

The instrument is now completely set up and ready to automatically run the IDF/SDF procedure. Press the “START” button to begin. At the beginning of each new run, the instrument automatically runs a tube integrity test.

For the first run after a power-up cycle, or for the first run after the instrument has been idle for twelve hours, the following status screen will be displayed.



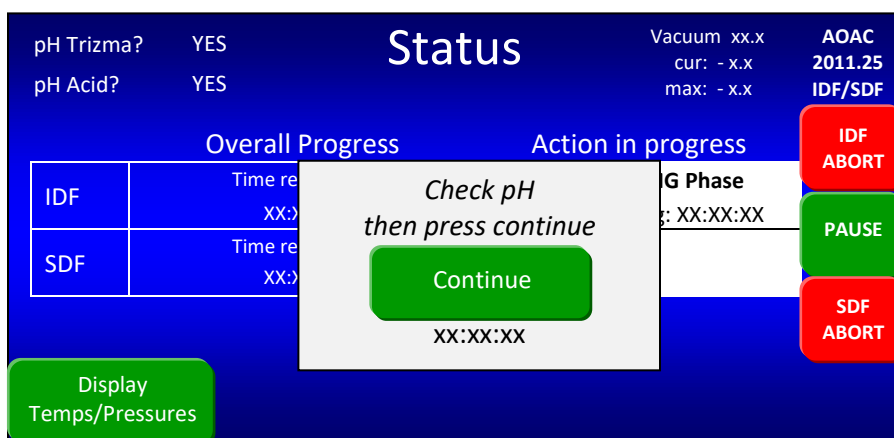
If the fluid lines are already charged properly, or when the line charge is complete, the following screen will be displayed.



When the tube integrity check is complete, the instrument will automatically execute the 16-hour 50-minute digestion, the IDF filtration, the 60-minute precipitation, and the SDF filtration, adding all solutions as needed. The instrument will only stop for faults, aborts, manual pH measurement (if enabled), and internal standard addition (if enabled). The Status screen will show actions and faults as they occur during the automatic operation.

23. Manually measure pH after Trizma base addition.

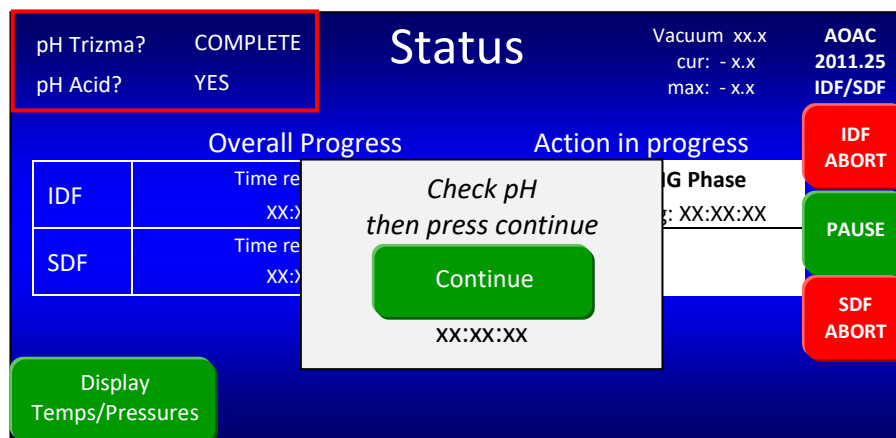
You are asked before starting the automated procedure if you want to check pH after the Trizma base addition. If you answered “Yes” to this question, the instrument will stop after adding the required Trizma, open Clamp Bar A, display the screen below, and make a sound to remind you to manually measure pH and adjust to 7.9 - 8.4 if necessary.



When measuring pH use a probe that can be easily rinsed (with DI or DW water) to avoid loss of sample. If you add acid or base to adjust the pH, you must mix the solution in order to get an accurate pH reading. To manually mix the solution, press the outside of the bag with your fingers just above Clamp Bar B multiple times.

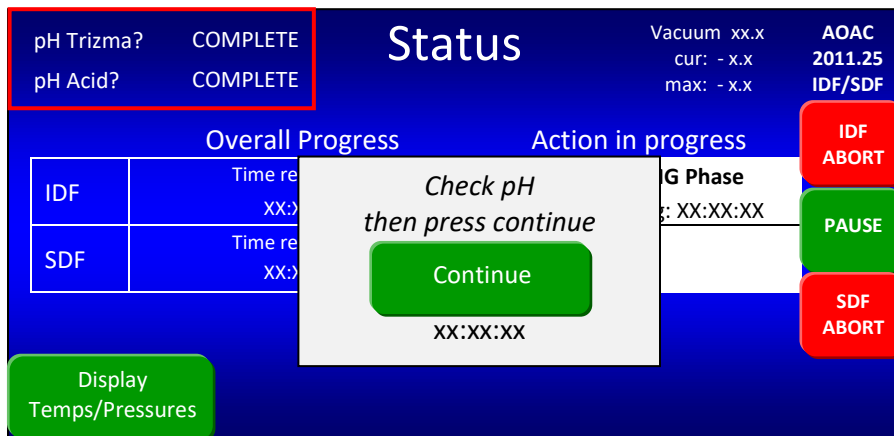


When you have completed the pH measurement process straighten each bag by lifting them back into place. Press the “Continue” button on the Status screen. You will see “COMPLETE” next to “pH Trizma?” in the upper left corner of the screen.



24. Manually measure pH after Acetic acid addition.

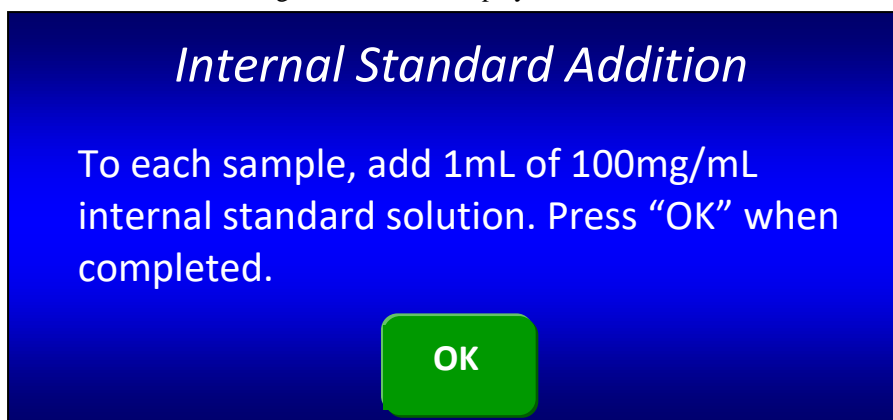
You are asked before starting the automated procedure if you want to check pH after the Acetic acid addition. If you answered “Yes” to this question, the instrument will stop after adding the required Acetic acid, open Clamp Bar A, display the screen below, and make a sound to remind you to manually measure pH and adjust to approximately 4.3 if necessary.



When you have completed the pH measurement process, press the **Continue** button on the Status screen. You will see “COMPLETE” next to “pH Acid?” in the upper left corner of the screen.

25. Add Internal Standard (if not done during set-up).

If you did not add Internal Standard during the instrument set-up, after the instrument adds the Acetic acid and any manual pH modifications are made, the following screen will be displayed.



Add Internal Standard to the IDF bag; then press the “OK” button.

26. Rinse the IDF filter bags with acetone.

After the instrument has completed an IDF process the IDF residue that has been collected in the IDF bag must be manually rinsed twice with acetone. It is recommended that you use an ANKOM TDF51 Rinse Stand (sold separately) for rinsing filter bags with acetone.

To rinse the IDF filter bags with acetone using the ANKOM TDF51 Rinse Stand, follow the steps below.

- 26.1 Remove the IDF bags from the instrument.
- 26.2 Place the bags on the Rinse Stand by sliding the back part of each bag under the pinch mechanism. Keep the top of the bag open.



- 26.3 Using a wash bottle, squirt acetone completely around the inside polypropylene surfaces of each bag, making sure that all residue is rinsed down into the filter.
- 26.4 Total of two times.
- 26.5 Allow acetone to evaporate from the bags for 30-40 minutes.



Hazardous Materials – Do NOT heat seal or place bags in an oven until all acetone has evaporated.

- 26.6 With your Heat Sealer set between 3 and 4 (settings may vary depending on the heat sealer and the power source), press the Heat Sealer arm down. **Hold the arm down for 3 to 4 seconds after the light goes out** to seal each bag just above the filter. This keeps all residue contained to the filter area while handling the bags.



- 26.7 Place each bag in the Drying Rack.



27. Rinse the SDF filter bags with acetone.

After the instrument has completed an SDF process the SDF residue that has been collected in the SDF bag must be manually rinsed with acetone. It is recommended that you use an ANKOM TDF51 Rinse Stand for rinsing filter bags with acetone.

To rinse the SDF filter bags with acetone using the ANKOM TDF51 Rinse Stand, follow the steps below.

- 27.1 Remove the SDF bags from the instrument.
- 27.2 Place the bags on the Rinse Stand by sliding the back part of each bag under the pinch mechanism. Keep the top of the bag open.
- 27.3 Using a wash bottle, squirt acetone into each bag, making sure that all residue on the clear polypropylene surfaces is rinsed down into the filter.
- 27.4 Repeat the previous step so that each bag gets rinsed a total of two times.
- 27.5 Allow acetone to evaporate from the bags.



Hazardous Materials – Do NOT heat seal or place bags in an oven until all acetone has evaporated.

- 27.6 Seal each bag just above the filter to keep all residue contained to the filter area while handling the bags.

NOTE:

Seal each bag as close to the filter as possible. This will allow you to trim the maximum amount of polypropylene from the bag before determining the protein content.

- 27.7 Place each bag in the Drying Rack.

28. Dry the IDF and SDF filter bags.

- 28.1 Make sure your oven is 105°C at the location where the bags will be placed.
- 28.2 Place the Drying Rack with the filter bags in the oven and dry to constant weight (a minimum of 90 minutes).
- 28.3 When dry, remove all IDF and SDF filter bags from the oven and place them in separate desiccant pouches (X45).

29. Weigh the IDF residue.

- 29.1 Remove only one IDF filter bag from the desiccant pouch at a time. (Close the desiccant bag after each bag is removed).
- 29.2 Roll or fold the bag, place in a tared Bag Weigh Holder, and place the Bag Weigh Holder in the center of a scale.
- 29.3 Record the weight of the filter bag.
- 29.4 Repeat the previous steps for each IDF filter bag used in the fiber analysis.

30. Weigh the Water Soluble, Water: Alcohol Insoluble Dietary Fiber (WSWAIDF) residue.

- 30.1 Remove only one SDF filter bag from the desiccant pouch at a time. (Close the desiccant bag after each bag is removed).
- 30.2 Roll or fold the bag, place in a tared Bag Weigh Holder, and place the Bag Weigh Holder in the center of a scale.
- 30.3 Record the weight of the filter bag.
- 30.4 Repeat the previous steps for each SDF filter bag used in the fiber analysis.

31. Determine the Protein content within the IDF and WSWAIDF residue.

See the “Protein Determination” section of the TDF Operator’s Manual for recommended procedures.

32. Determine the Ash content within the IDF and WSWAIDF residue.

See the “Ash Determination” section of the TDF Operator’s Manual for recommended procedures.

33. Determine the Water: Alcohol Soluble dietary fiber (WASDF)

Using the filtrate in the instrument Filtrate Cups, determine the WASDF according to the approved method.

34. Calculate the % IDF value.

% IDF		=	$\left[\frac{[(R_1 + R_2)/2] - P - A - B}{(M_1 + M_2)/2} \right] \times 100$
		=	$\left[\frac{[((f_{F1} - f_{S1}) + (f_{F2} - f_{S2}))/2] - P - A - B}{(M_1 + M_2)/2} \right] \times 100$
Where:	M ₁ , M ₂	=	Original wt of duplicate samples adjusted for pre-treatment fat and sugar losses (g)
	R ₁ , R ₂	=	Residue for duplicate samples (g)
	f _F	=	Final Filter Bag with residue (g)
	f _S	=	Initial Filter Bag (g)
	P	=	Protein of residue and bag (g)
	A	=	Ash of residue and bag (g)
	B	=	Blank (g)
		=	$[(BR_1 + BR_2)/2] - P_B - A_B$
		=	$[(f_{BF1} - f_{BS1}) + (f_{BF2} - f_{BS2}))/2] - P_B - A_B$
	BR ₁ , BR ₂	=	Residue for duplicate blanks (g)
	f _{BF}	=	Final Blank Filter Bag (g)
	f _{BS}	=	Initial Blank Filter Bag (g)
	P _B	=	Protein of Blank Filter Bag (g)
	A _B	=	Ash of Blank Filter Bag (g)

35. Calculate the % SDF value.

% SDF	=	% WSWAIDF + % WASDF
	=	$\left(\left[\frac{[(R_1 + R_2)/2] - P - A - B}{(M_1 + M_2)/2} \right] \times 100 \right) + \% \text{ WASDF}$
	=	$\left(\left[\frac{[(f_{F1} - f_{S1} - D_1) + (f_{F2} - f_{S2} - D_2)]/2 - P_1 - (A_2 - D_2) - B}{(M_1 + M_2)/2} \right] \times 100 \right) + \% \text{ WASDF}$
Where:	M ₁ , M ₂	= Original wt of duplicate samples adjusted for pre-treatment fat losses (g)
	R ₁ , R ₂	= Residue for duplicate samples (g)
	f _F	= Final Filter Bag with residue (g)
	f _S	= Initial Filter Bag (g)
	D	= Original wt of Diatomaceous Earth (g)
	P	= Protein of residue and bag (g)
	A	= Ash of residue and bag (g)
	B	= Blank (g)
		= [(BR ₁ + BR ₂)/2] - P _B - (A _B - D _B)
		= [(f _{BF1} - f _{BS1} - D _{B1}) + (f _{BF2} - f _{BS2} - D _{B2})]/2 - P _{B1} - (A _{B2} - D _{B2})
	BR ₁ , BR ₂	= Residue for duplicate blanks (g)
	f _{BF}	= Final Blank Filter Bag (g)
	f _{BS}	= Initial Blank Filter Bag (g)
	P _B	= Protein of Blank Filter Bag (g)
	A _B	= Ash of Blank Filter Bag (g)
	D _B	= Original wt of Diatomaceous Earth in Blank Filter Bag (g)
	WASDF	= Soluble Dietary Fiber determined by HPLC

36. Calculate the % TDF value by adding the % IDF and the % SDF values.

37. Crystallization of TRIZMA Base in the silicone tubing can happen overnight and will cause a fault during a run the next day. To prevent this, it is necessary to flush this line out at the end of each day that the instrument has been used. Please see Appendix C for the flush procedure.

TDF Analysis (AOAC 2009.01)

A TDF analysis directly measures the amount of TDF within a given sample without measuring the IDF and SDF fractions. This analysis requires a filter bag for the precipitation process and a non-filter IDF *Flow-Thru* bag for the digestion process. When starting a new run, the ANKOM^{TDF} Dietary Fiber Analyzer must have bags installed at all stations (a total of twelve bags).

NOTE:

To enhance the productivity of your instrument, you can begin the IDF process for a new run while the SDF process of a previous run is finishing. See the “Productivity Enhancement” section of your ANKOM^{TDF} Dietary Fiber Analyzer Operator’s Manual for more details.

To perform a TDF analysis, follow the steps below.

1. Prepare chemicals and enzymes.

When using the ANKOM^{TDF} Dietary Fiber Analyzer for the AOAC 2009.01 method, use the chemicals and enzymes referred to therein. See Appendix A of this manual for the list of chemicals and enzymes and the instructions for how to prepare them for use in this instrument.

IMPORTANT:

In order to prevent any TRIZMA base from crystalizing in the silicone tubing and causing

2. For samples that have a fat content of 10% or higher, de-fat your samples as needed according to the official methods or your internal SOPs.

3. Label the filter bags using a Solvent Resistant Marker.

IDF *Flow-thru* Bag
(shorter bag no filter)



SDF Bag
(longer bag with filter)

4. Prepare for data collection.

You will need a place to store the data collected during this analysis. For your convenience a CD titled “Calculation Templates” is included with the instrument. This CD has Data Spreadsheets that can be used for this analysis. Please read the “Instructions” tab in the MS-Excel file for information about the spreadsheets.

5. Weigh SDF bags.

IMPORTANT:

Using a Bag Weigh Holder is critical to eliminate the effects of static electricity during the weighing process.

Roll or fold each bag and place it in a tared Bag Weigh Holder. Place the Bag Weigh Holder in the center of a balance and record the weight.



NOTE:

Because different balances have different sensitivities, the Bag Weigh Holder should be placed in the center of the balance for best results.

6. Weigh Diatomaceous Earth (DE).

DE is used during fiber analysis to enhance the flocculation and filtration of the SDF fraction. Place ca 1g \pm 0.05g of DE in each of six tared and numbered tins and record the weights.

7. Weigh Samples.

Place 0.5 \pm 0.05 g of sample in each of six tared and numbered tins and record the weights.

NOTE:

A larger sample (1.0g) can be used to improve precision if necessary.

8. Confirm Nitrogen Supply and Pressure Ranges.

Make sure the Nitrogen supply in your lab is connected to the instrument and turned on. The High-Pressure Gauge on the left of the instrument should be set between 50-55 psi. The Low-Pressure Gauge on the right side of the instrument should be set at 4 psi. The accuracy of the Low-Pressure Gauge should be checked prior to each run by pressing the “Pressurize SDF” button on the control panel twice.

High Pressure Gauge
50-55 psi (3.5-3.8 bar)



Low Pressure Gauge
4 psi (0.3 bar)

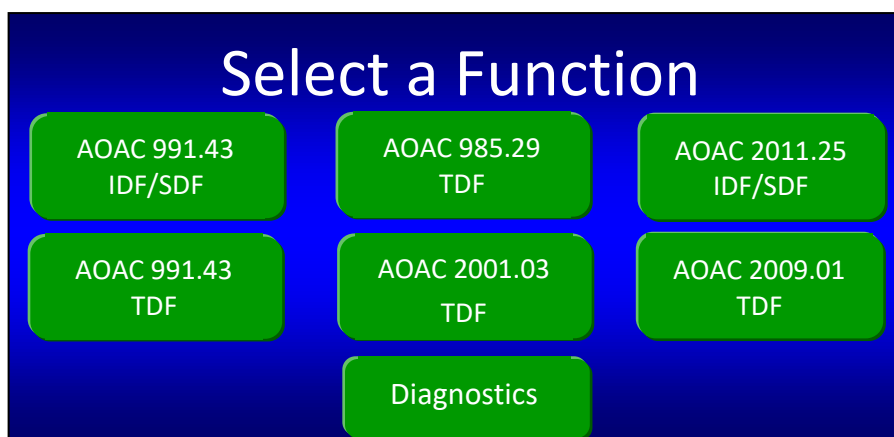
**9. Turn the instrument power on.**

When you turn the power on, the instrument will run through an initialization process and the Control Panel will turn on.

10. On the Touch Screen Display, select the instrument function you would like to perform.

The Control Panel on the ANKOM^{TDF} Dietary Fiber Analyzer uses Touch Screen technology. To operate the instrument, you will press identified buttons on the Touch Screen Display and buttons below the screen.

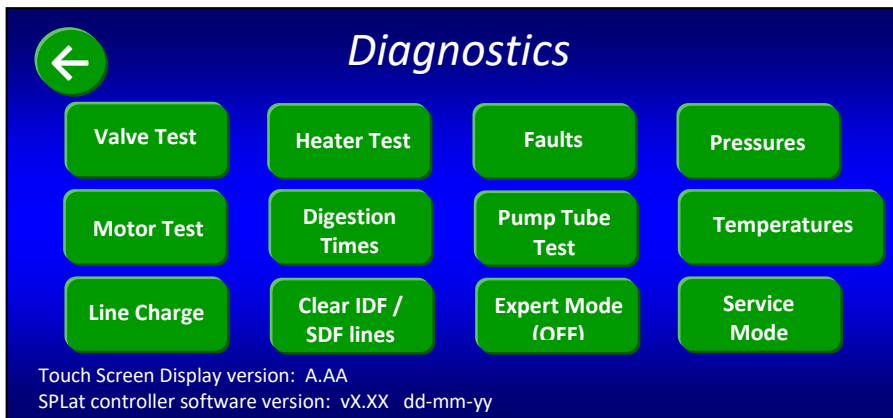
When your instrument is initialized and ready to operate, the following screen will be displayed.

**NOTE:**

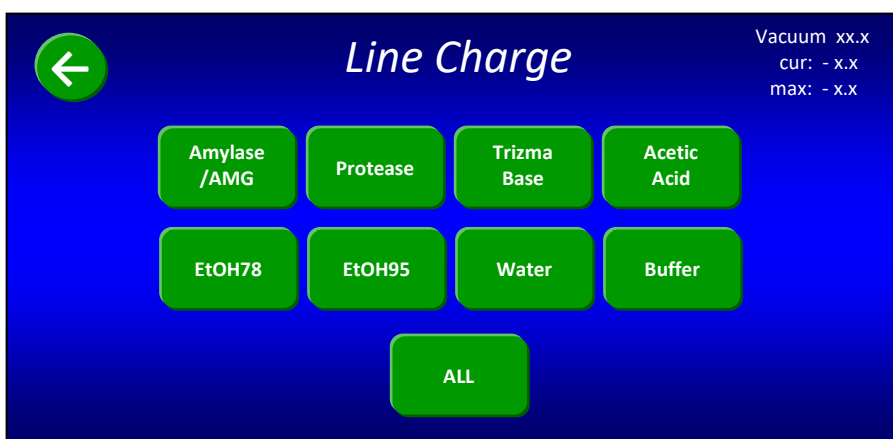
Although the instrument works in accordance with the AACC 32-45.01 method, the Touch Screen Display only refers to the AOAC methods.

- 10.1 If this is the first time the instrument is being operated after being installation, or if this is the first time the instrument is being operated after sitting unused for a period of time and was flushed according to Appendix C, the lines must be charged to avoid the faults: E13 (Tubing Failure) or E14 (Empty Chemical Container). To charge the lines follow the steps below.


10.1.1 Press the “Diagnostics” button on the “Select a Function” screen. The following screen will be displayed.

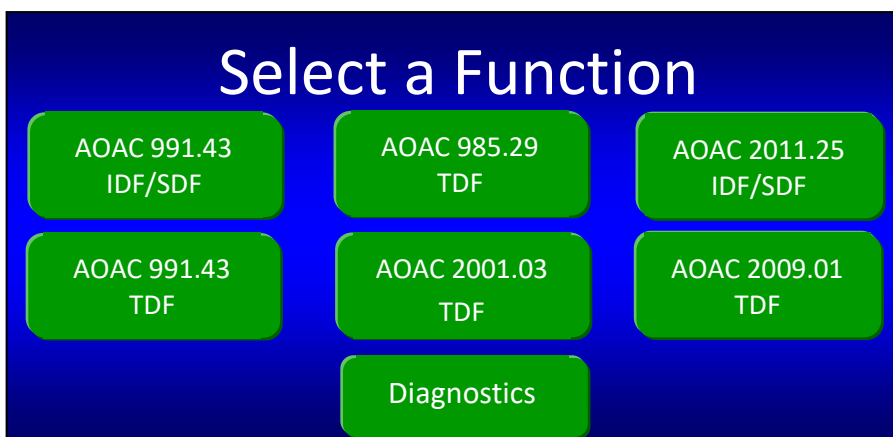


10.1.2 Press the “Line Charge” button. The following screen will be displayed.

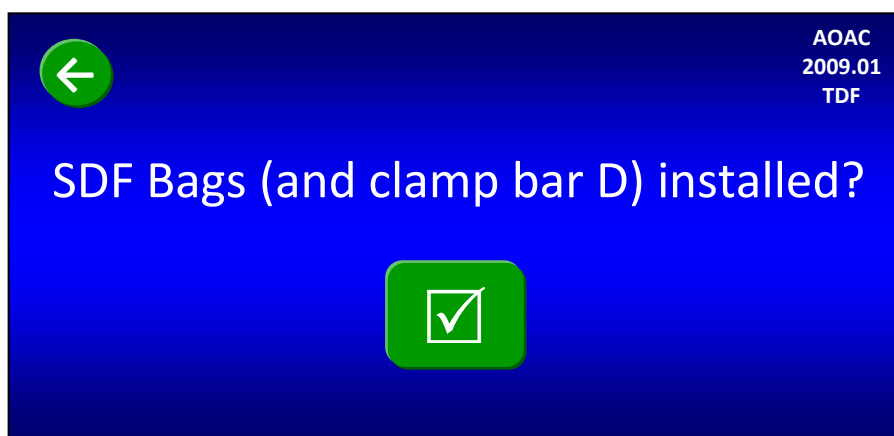



10.1.3 Press the “ALL” button to charge all of the lines. Each button will change color as the associated line is being charged. All lines are charged when all of the buttons return to their original green color.

10.1.4 Press the back  button twice. The following “Select a Function” screen will be displayed again.



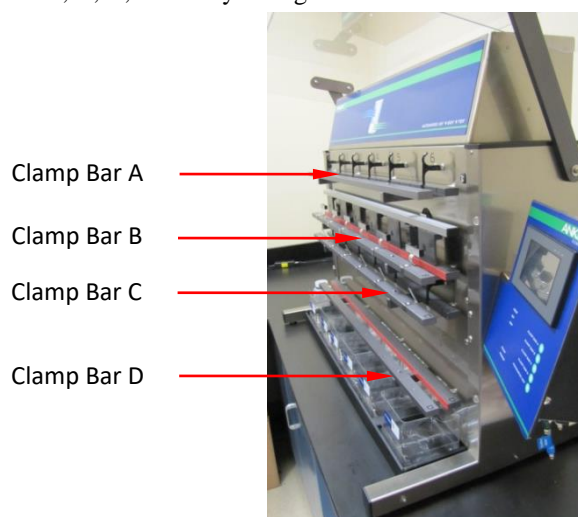
- 10.2 Press the “AOAC 2009.01 TDF” button. The following screen will be displayed.



Notice that the function you selected is now displayed in the top right corner of the screen and a back  button is displayed in the top left corner of the screen.

11. Install SDF filter bags on the ANKOM^{TDF} Dietary Fiber Analyzer.

- 11.1 Remove Clamp Bars A, B, C, and D by lifting them off of the locator rods.



- 11.2 Gently pull the black SDF Delivery Nozzle out toward you.



- 11.3 Place a labeled and weighed SDF bag up underneath the SDF Delivery Nozzle so that the Delivery Nozzle is inside the top part of the bag. Pull the bag up so that the top of the bag is about 35 mm (1.375 inches) above the top of Clamp Bar C and return the Delivery Nozzle to its original position. This will hold the back of the bag in place.



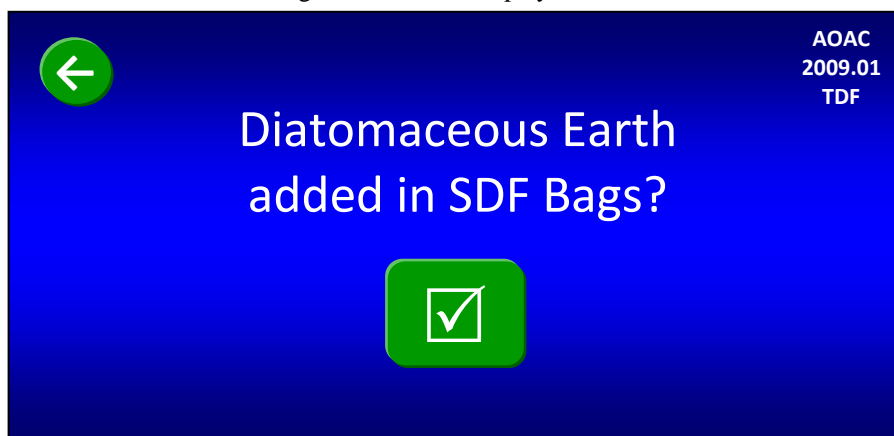
IMPORTANT:

As part of normal operation, solution from the IDF *Flow-thru* bag will flow into the SDF bag. Therefore, when installing the SDF filter bags it is very important to position them high enough vertically so that at least 20 mm (0.75 inches) of the bottom of the IDF *Flow-thru* bag can fit inside the top of the SDF bag.

- 11.4 With the bag being held by the Delivery Nozzle, center it horizontally between the lines on the back part of Clamp Bar C.



- 11.5 Repeat the previous steps for all six stations.
- 11.6 Re-install Clamp Bar D by setting it on the locator rods. Make sure the letter is on the top of the bar and the rubber material is facing in toward the instrument.
- 11.7 Flatten the bags to remove any wrinkles.
- 11.8 With fingers away from the clamp bars, press the check mark (☑) button on the Touch Screen Display to pinch the bags just above the filter. The following screen will be displayed.



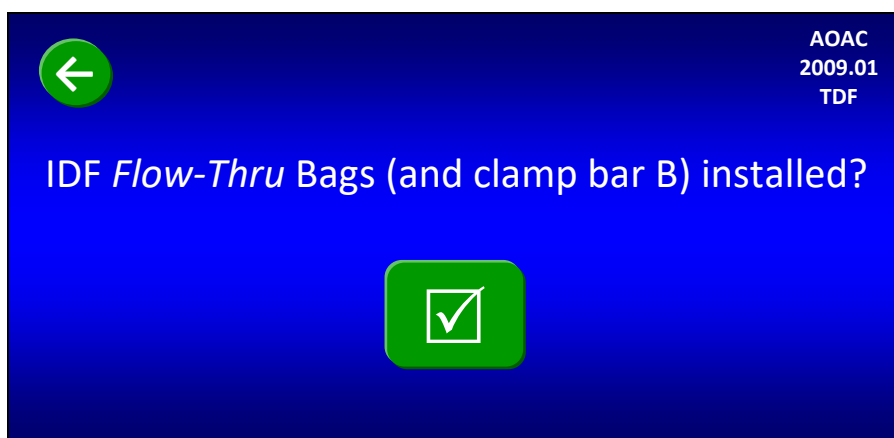
12. Add DE to the SDF filter bags on the ANKOM^{TDF} Dietary Fiber Analyzer.

- 12.1 Open the top of the SDF bag and add a weigh tin of DE to the bag by folding the tin and then dipping it down into the bag below the tip of the Delivery Nozzle.



IMPORTANT: When adding DE to the filter bags it is very important to keep it below the tip of the Delivery Nozzle so that the DE material can be properly rinsed.

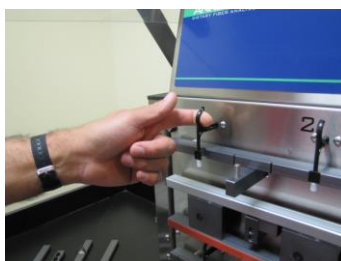
- 12.2 Rinse the weigh tin/weigh boat 78% EtOH to ensure complete transfer.
12.3 Repeat the previous steps for all six stations.
12.4 Press the check mark (✓) button on the Touch Screen Display. The following screen will be displayed.



NOTE: After you confirm that the DE is added, the Clamp Bar D button on the Control Panel is disabled until the SDF process completes.

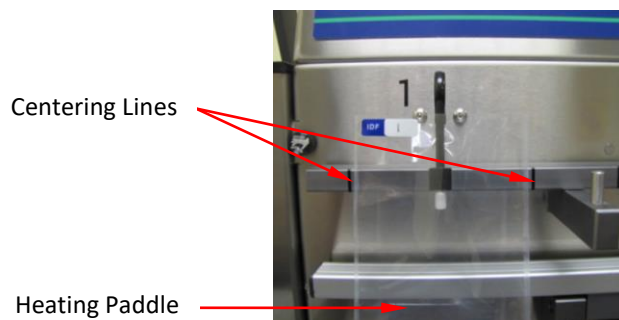
13. Install IDF *Flow-thru* bags on the ANKOM^{TDF} Dietary Fiber Analyzer.

- 13.1 Gently pull the black IDF Delivery Nozzle out toward you.



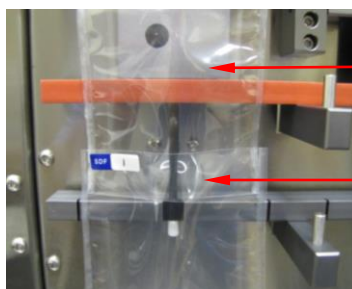
- 13.2 Place a labeled IDF *Flow-Thru* bag up underneath the Delivery Nozzle so that the Delivery Nozzle is inside the top part of the bag. Pull the bag up so that the top of the bag is about 35 mm (1.375 inches) above the top of Clamp Bar A and return the Delivery Nozzle to its original position. This will hold the back of the bag in place.

- 13.3 With the bag held by the Delivery Nozzle, center it horizontally between the Centering Lines on the back part of Clamp Bar A.



IMPORTANT: For proper mixing during the IDF process the IDF *Flow-thru* bags must be horizontally centered over the Heating Paddles and between the Centering Lines on the back part of Clamp Bar A.

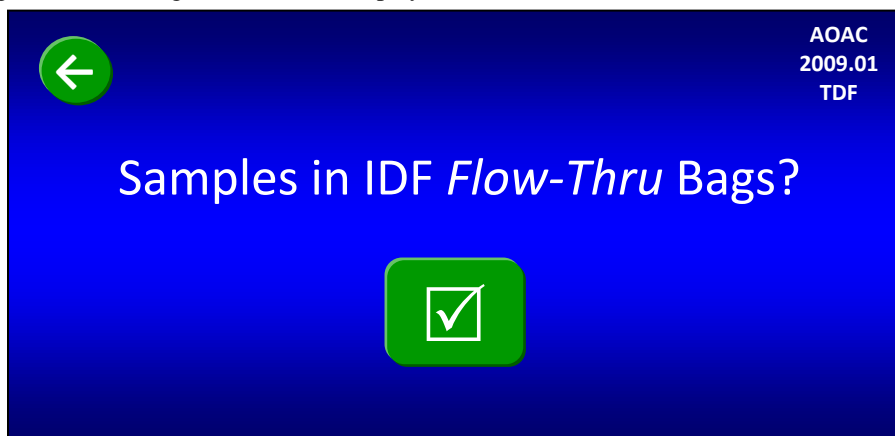
- 13.4 Place at least 20 mm (0.75 inches) of the bottom of the IDF *Flow-Thru* bag inside the top of the SDF bag to allow for the flow of solution into the SDF bag after the digestion process is complete.



Heating Paddle

20 mm (0.75 inches) of the
IDF *Flow-Thru* bag inside
the top of the SDF bag

- 13.5 Repeat the previous steps for all six stations.
- 13.6 Re-install Clamp Bar B by setting it on the locator rods. Make sure the letter is on the top of the bar and the rubber material is facing in toward the instrument.
- 13.7 Flatten the IDF *Flow-Thru* bags to remove any wrinkles.
- 13.8 With fingers away from the clamp bars, press the check mark (☑) button on the Touch Screen Display to pinch the IDF *Flow-Thru* bags. The mixing pads will make contact with the bags when Clamp Bar B pinches the IDF *Flow-Thru* bags. The following screen will be displayed.



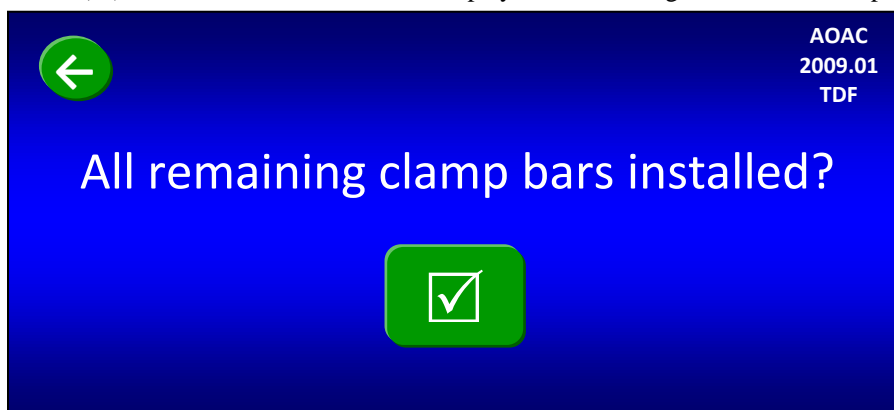
14. Add samples to the IDF *Flow-thru* bags.

- 14.1 Open the top of the IDF *Flow-thru* bag and transfer the sample from a weigh tin into the bag by folding the tin and then dipping it down into the bag below the tip of the Delivery Nozzle.

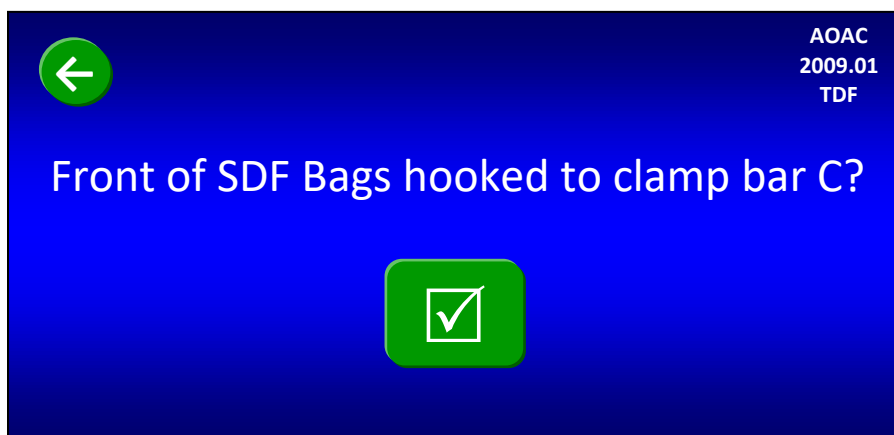


IMPORTANT: When adding sample to the IDF *Flow-thru* bags it is very important to keep it below the tip of the Delivery Nozzle so that it can be properly rinsed.

- 14.2 Rinse the tin with no more than 3 ml of DI or DW water to ensure complete transfer.
- 14.3 Repeat the previous steps for all IDF stations.
- 14.4 Re-install Clamp Bar A by setting it on the locator rods. Make sure the letter is on the top of the bar and the rubber material is facing in toward the instrument.
- 14.5 Press the check mark (☑) button on the Touch Screen Display. The following screen will be displayed.



- 14.6 Make sure that all clamp bars are installed with the letter on the top of the bar and the rubber material facing in toward the instrument. Press the check mark (☑) button on the Touch Screen Display. The following screen will be displayed.



15. Hook the front of the SDF bag in place.

- 15.1 Secure the SDF filter bags in place with the hooks located on the front part of Clamp Bar C by pulling up the middle section of the front of the filter bag evenly and tightly, lean the bag against the hook as the bag is lowered, allowing the bag to catch onto the hook. There should be at least a 1/4" of the top of the bag sitting above the hook.

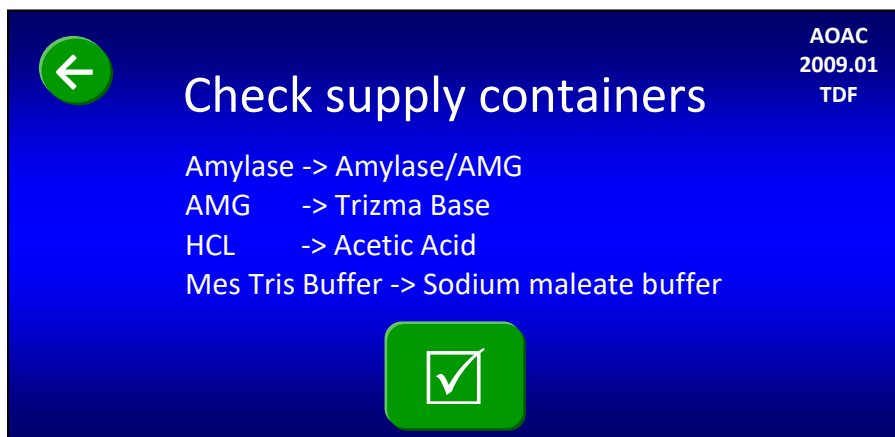


Hook on front part of
Clamp Bar C

NOTE:

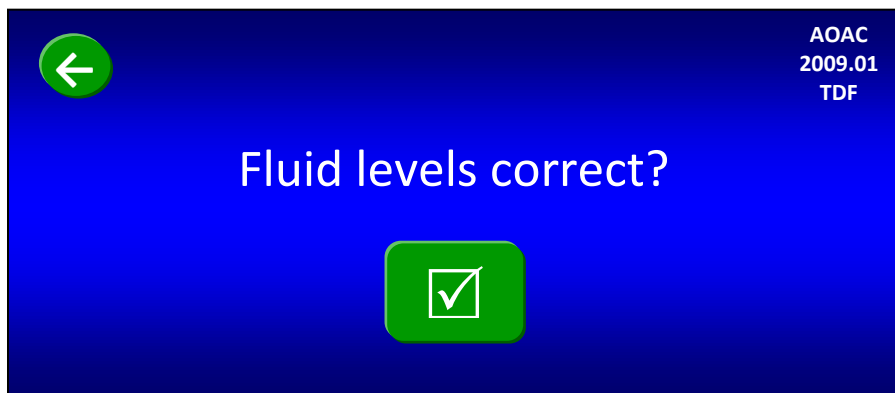
Hooking the bags in place will put a physical hole in the front of each SDF bag.

- 15.2 Press the check mark (☑) button on the Touch Screen Display. The following screen will be displayed.



16. Verify that the fluid containers are correct for this procedure.

Verify that the fluid supply containers are configured according to the screen above and press the check mark (☑) button. The following screen will be displayed.



17. Fill fluid containers.

To ensure that you have enough fluids to run a complete TDF procedure, you must begin with at least 30 ml of the Amylase/AMG mixture, 15 ml of Protease, 30 ml of Trizma Base, and fluid levels above the Minimum Level lines on the remaining chemical containers. Add fluids and enzymes as necessary.

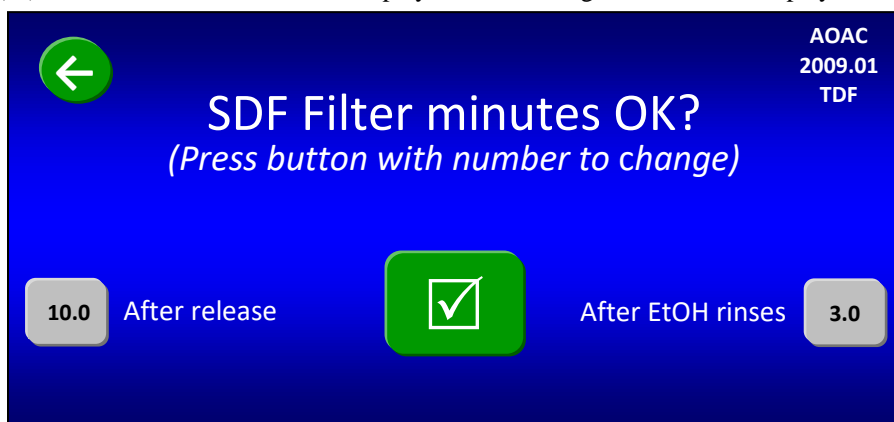
With all fluid containers filled to the proper levels, press the check mark (☑) button on the Touch Screen Display. The following screen will be displayed.



18. Connect the Nitrogen Supply to the instrument and turn on.

Make sure the Nitrogen supply in your lab is connected to the instrument and turned on. The pressure gauges on the instrument should show 50-55 psi on the left and about 4 psi on the right.

Press the check mark (☑) button on the Touch Screen Display. The following screen will be displayed.



19. Set filter times (in minutes).

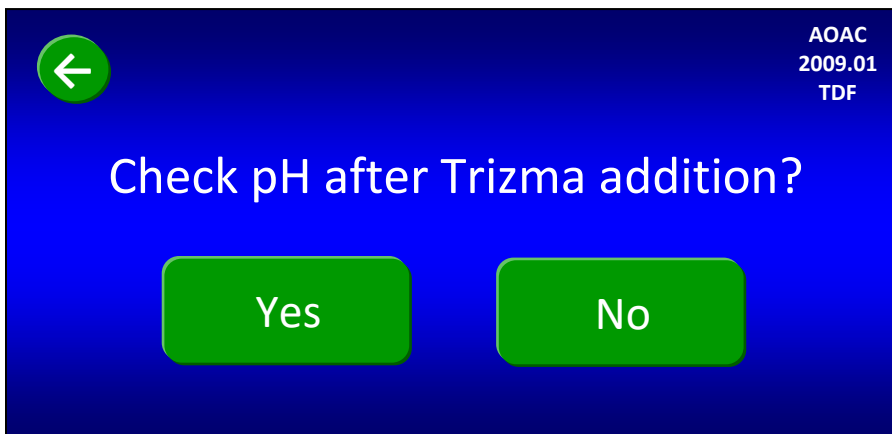
Because different samples take different amounts of time to filter, the above screen allows you to set your filter times. To change any of the times shown on the screen, press the specific gray button. A number pad will be displayed that will allow you to enter the time that you want. The times you enter will remain until you change them again.

NOTE:

The initial filter times shown when you first run the instrument are based on factory experience. During filtration the computer allows you to bypass the filter time if you notice the filtering is complete. The computer also allows you to add time during filtering if needed (see the "Status Screen" section of the Operator's Manual for more detail).

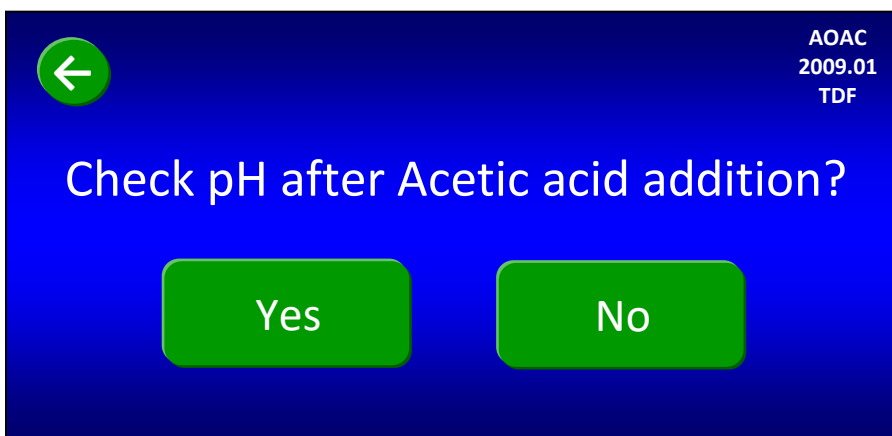
When all of the times shown on the screen are what you want, press the check mark (☑) button on the Touch Screen

Display. The following screen will be displayed.

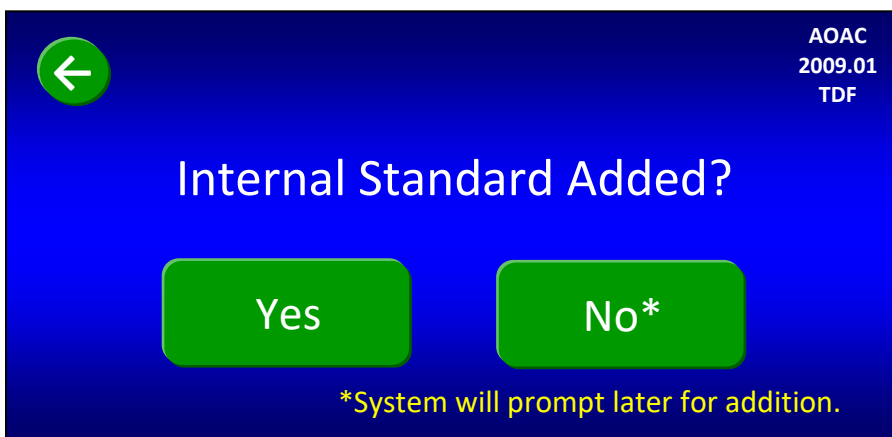


20. Set the Manual pH checks.

If you plan to check the pH after the required Trizma base is added (during the IDF process), press the “Yes” button on the screen above. Otherwise, press the “No” button. The following screen will be displayed.

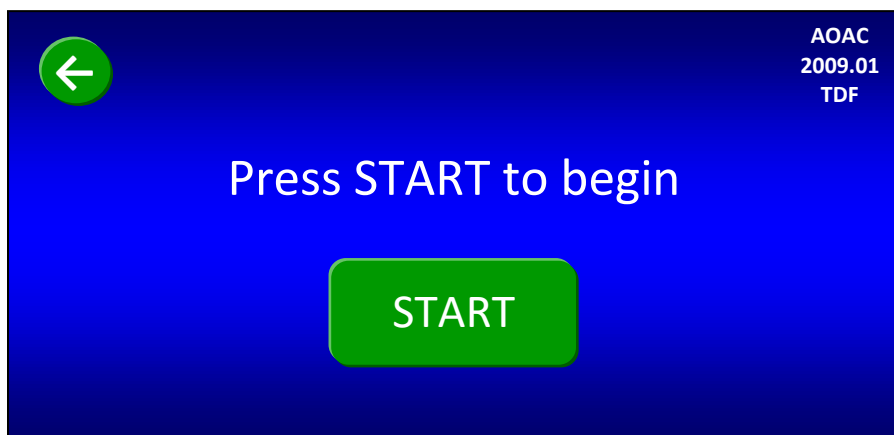


If you plan to check the pH after the required Acetic acid is added (during the IDF process), press the “Yes” button on the screen above. Otherwise, press the “No” button. The following screen will be displayed.



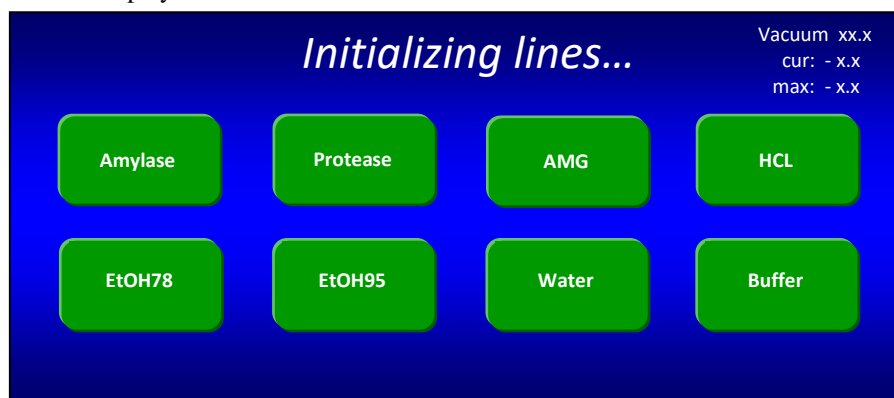
21. Add Internal Standard now or tell the instrument to prompt you later.

You can either add 1 ml of the Internal Standard (as per the AOAC 2009.01 method) before starting the instrument, or you can be prompted to add it after the pH checks. If you want to add it before starting the instrument, add it now to each IDF *Flow-thru* bag and press the “Yes” button. If you want to add it after the pH checks (during the IDF process), press the “No” button on the screen above and the instrument will prompt for the addition later. The following screen will be displayed.

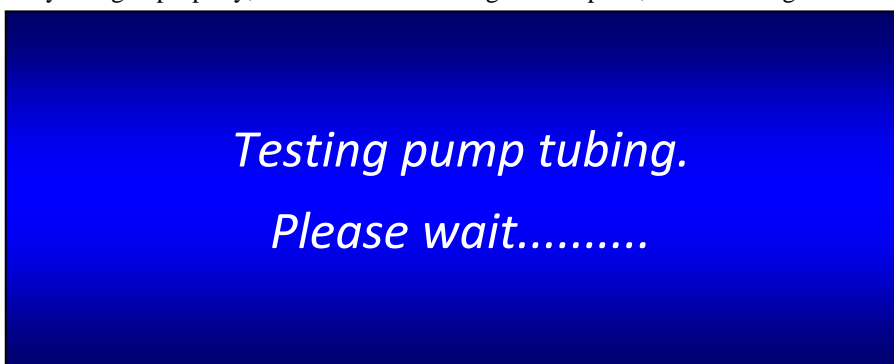
**22. Start the instrument.**

The instrument is now completely set up and ready to automatically run the TDF procedure. Press the “START” button to begin. At the beginning of each new run, the instrument automatically runs a tube integrity test.

For the first run after a power-up cycle, or for the first run after the instrument has been idle for twelve hours, the following status screen will be displayed.



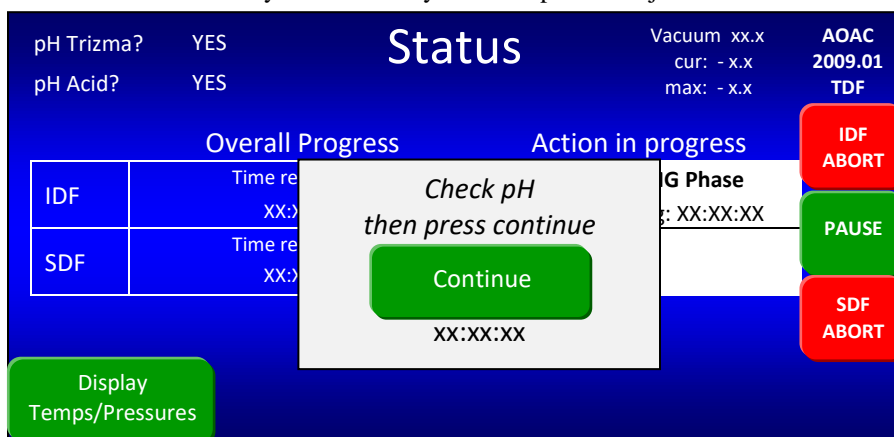
If the fluid lines are already charged properly, or when the line charge is complete, the following screen will be displayed.



When the tube integrity check is complete, the instrument will automatically execute the 16-hour 50-minute digestion, the 60-minute precipitation, and the filtration, adding all solutions as needed. The instrument will only stop for faults, aborts, manual pH measurement (if enabled), and internal standard addition (if enabled). The Status screen will show actions and faults as they occur during the automatic operation.

23. Manually measure pH after Trizma base addition.

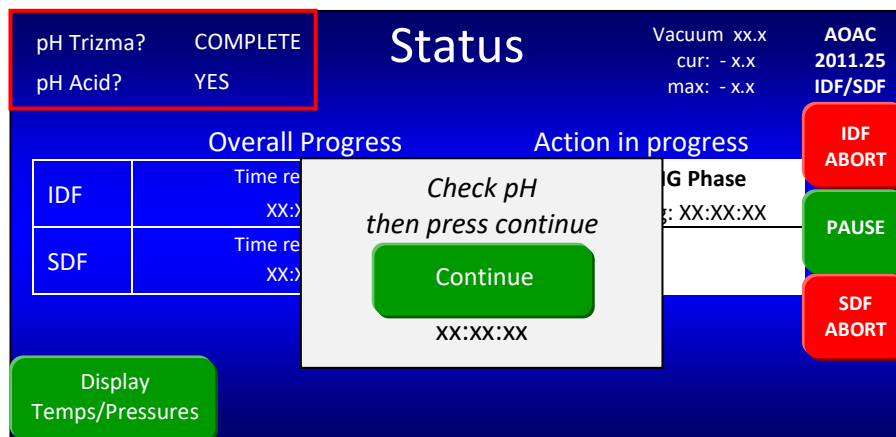
You are asked before starting the automated procedure if you want to check pH after the Trizma base addition. If you answered “Yes” to this question, the instrument will stop after adding the required Trizma, open Clamp Bar A, display the screen below, and make a sound to remind you to manually measure pH and adjust to 7.9 - 8.4 if necessary.



When measuring pH use a probe that can be easily rinsed (with DI or DW water) to avoid loss of sample. If you add acid or base to adjust the pH, you must mix the solution in order to get an accurate pH reading. To manually mix the solution, press the outside of the bag with your fingers just above Clamp Bar B multiple times.

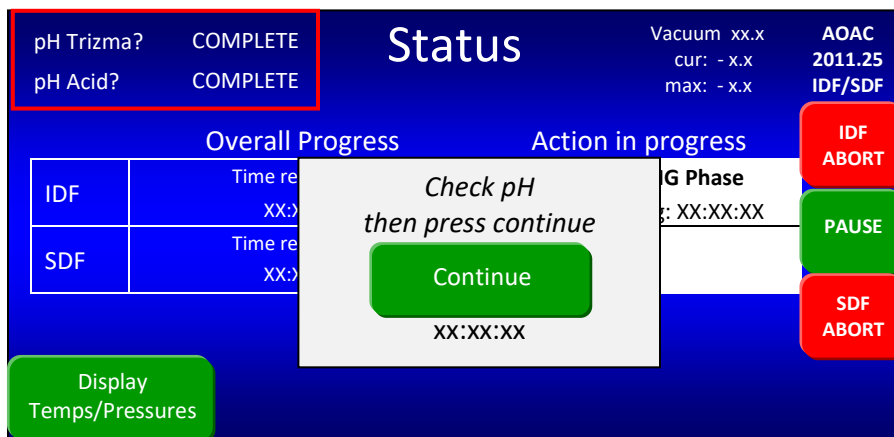


When you have completed the pH measurement process straighten each bag by lifting them back into place. Press the “Continue” button on the Status screen. You will see “COMPLETE” next to “pH Trizma?” in the upper left corner of the screen.



24. Manually measure pH after Acetic acid addition.

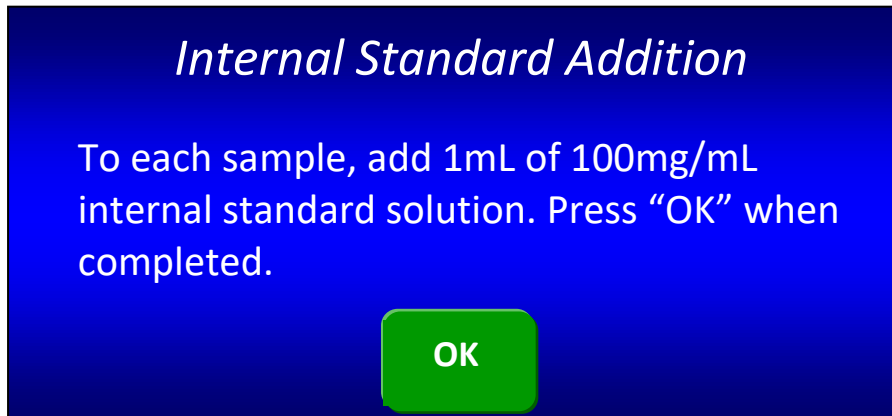
You are asked before starting the automated procedure if you want to check pH after the Acetic acid addition. If you answered “Yes” to this question, the instrument will stop after adding the required Acetic acid, open Clamp Bar A, display the screen below, and make a sound to remind you to manually measure pH and adjust to approximately 4.3 if necessary.



When you have completed the pH measurement process straighten each bag by lifting them back into place. Press the “Continue” button on the Status screen. You will see “COMPLETE” next to “pH Acid?” in the upper left corner of the screen.

25. Add Internal Standard (if not done during set-up).

If you did not add Internal Standard during the instrument set-up, after the instrument adds the Acetic acid and any manual pH modifications are made, the following screen will be displayed.



Add Internal Standard to the IDF *Flow-Thru* bag; then press the “OK” button.

26. Make sure that each sample is completely rinsed down into the SDF filter bags (as needed).

Since the IDF *Flow-thru* bags are not weighed before and after the process, it is important to make sure that each sample gets rinsed down to the SDF filter bag when the digestion process is complete. This is especially important for sticky samples.

If you notice sample sticking to an IDF *Flow-thru* bag, use 78% EtOH in a wash bottle to rinse as needed.

27. Rinse the SDF filter bags with acetone.

After the instrument has completed a TDF process the TDF residue that has been collected in the SDF bag must be manually rinsed with acetone. It is recommended that you use an ANKOM TDF51 Rinse Stand for rinsing filter bags with acetone.

To rinse the SDF filter bags with acetone using the ANKOM TDF51 Rinse Stand, follow the steps below.

- 27.1 Remove the SDF bags from the instrument.
- 27.2 Place the bags on the Rinse Stand by sliding the back part of each bag under the pinch mechanism. Keep the top of the bag open.
- 27.3 Using a wash bottle, squirt acetone completely around the inside polypropylene surfaces of each bag two times, making sure that all residue is rinsed down into the filter.
- 27.4 Repeat the previous step so that each bag gets rinsed a total of two times.
- 27.5 Allow acetone to evaporate from the bags.



Hazardous Materials – Do NOT heat seal or place bags in an oven until all acetone has evaporated.

- 27.6 With your Heat Sealer set between 3 and 4 (settings may vary depending on the heat sealer and the power source), press the Heat Sealer arm down. **Hold the arm down for 3 to 4 seconds after the light goes out** to seal each bag just above the filter. This keeps all residue contained to the filter area while handling the bags.



NOTE:

Try to seal each bag as close to the filter as possible. This will allow you to trim the maximum amount of polypropylene from the bag before determining the protein content.

- 27.7 Place each bag in the Drying Rack.



28. Dry the SDF filter bags.

- 28.1 Make sure your oven is 105°C at the location where the bags are placed.
- 28.2 Place the Drying Rack with the filter bags in the oven and dry to constant weight (about 90 minutes).
- 28.3 When dry, remove all SDF filter bags from the oven and place them in separate desiccant pouches.

29. Weigh the Water Soluble, Water: Alcohol Insoluble Dietary Fiber (WSWAI) residue.

- 29.1 Remove only one SDF filter bag from the desiccant pouch at a time. (Close the desiccant bag after each bag is removed).
- 29.2 Roll or fold the bag, place it in a tared Bag Weigh Holder, and place the Bag Weigh Holder in the center of a scale.
- 29.3 Record the weight of the filter bag.
- 29.4 Repeat the previous steps for each SDF filter bag used in the fiber analysis.

30. Determine the Protein content within the WSWAIDF residue.

See the “Protein Determination” section of the TDF Operator’s Manual for recommended procedures.

31. Determine the Ash content within the WSWAIDF residue.

See the “Ash Determination” section of the TDF Operator’s Manual for recommended procedures.

32. Determine the Water: Alcohol Soluble dietary fiber (WASDF)

Using the filtrate in the instrument Filtrate Cups, determine the WASDF according to the approved method.

33. Calculate the % TDF value.

% TDF	=	% WSWAIDF + % WASDF
	=	$\left(\left[\frac{[(R_1 + R_2)/2] - P - A - B}{(M_1 + M_2)/2} \right] \times 100 \right) + \% \text{ WASDF}$
	=	$\left(\left[\frac{(((f_{F1} - f_{S1} - D_1) + (f_{F2} - f_{S2} - D_2))/2) - P_1 - (A_2 - D_2) - B}{(M_1 + M_2)/2} \right] \times 100 \right) + \% \text{ WASDF}$
Where:	M ₁ , M ₂	= Original wt of duplicate samples adjusted for pre-treatment fat losses (g)
	R ₁ , R ₂	= Residue for duplicate samples (g)
	f _F	= Final Filter Bag with residue (g)
	f _S	= Initial Filter Bag (g)
	D	= Original wt of Diatomaceous Earth (g)
	P	= Protein of residue and bag (g)
	A	= Ash of residue and bag (g)
	B	= Blank (g)
		= $[(BR_1 + BR_2)/2] - P_B - (A_B - D_B)$
		= $(((f_{BF1} - f_{BS1} - D_{B1}) + (f_{BF2} - f_{BS2} - D_{B2}))/2) - P_{B1} - (A_{B2} - D_{B2})$
	BR ₁ , BR ₂	= Residue for duplicate blanks (g)
	f _{BF}	= Final Blank Filter Bag (g)
	f _{BS}	= Initial Blank Filter Bag (g)
	P _B	= Protein of Blank Filter Bag (g)
	A _B	= Ash of Blank Filter Bag (g)
	D _B	= Original wt of Diatomaceous Earth in Blank Filter Bag (g)
	WASDF	= Soluble Dietary Fiber determined by HPLC

34. Crystallization of TRIZMA Base in the silicone tubing can happen overnight and will cause a fault during a run the next day. To prevent this, it is necessary to flush this line out at the end of each day that the instrument has been used. Please see Appendix C for the Trizma line Flush procedure.

Status Screen

The only Status Screen difference between the AOAC 991.43/985.29/2001.03 methods and the AOAC 2009.01/2011.25 methods is the pH information located in the upper left corner of the screen.

The screenshot shows the 'Status' screen with a blue background. In the top left corner, there are two input fields labeled 'pH Trizma?' and 'pH Acid?'. These fields are circled in red. To the right of these fields is the word 'Status' in large white text. Below the pH fields, there is a section titled 'Overall Progress' and 'Action in progress'. This section contains a table with two rows: 'IDF' and 'SDF'. Each row has a 'Time remaining:' field with a timer display 'XX:XX:XX'. To the right of this table are three buttons: 'IDF ABORT' (red), 'PAUSE' (green), and 'SDF ABORT' (red). At the bottom of the screen, there is a 'FAULT' button (red) and four other buttons: 'Display Temperatures' (green), 'Start New Procedure?' (green), 'Filter Time +' (green), and 'Filter Bypass' (green).

See the ANKOM^{TDF} Dietary Fiber Analyzer Operator's Manual for more information about the Status Screen.

Fault Handling

There are no Fault Handling differences between the AOAC 991.43/985.29/2001.03 methods and the AOAC 2009.01/2011.25 methods.

See the ANKOM^{TDF} Dietary Fiber Analyzer Operator's Manual for information about Fault Handling.

Diagnostics Mode

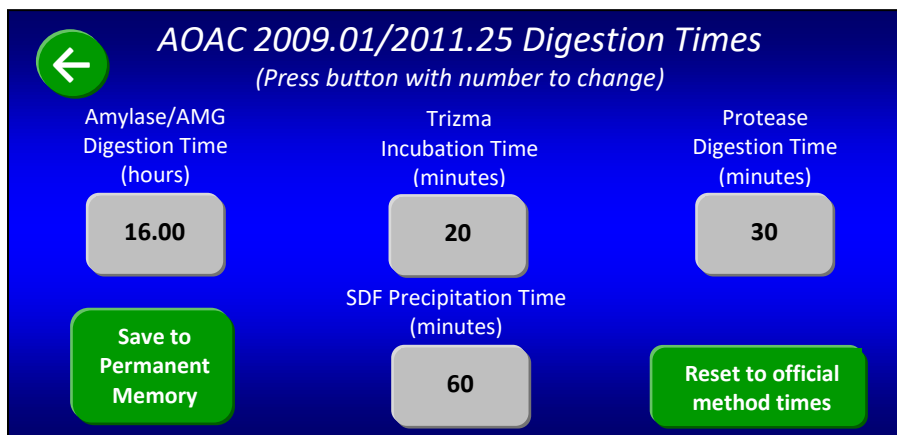
The only Diagnostics Mode differences between the AOAC 991.43/985.29/2001.03 methods and the AOAC 2009.01/2011.25 methods are shown below.

Digestion Times

When you press the "Digestion Times" button on the Diagnostics screen, you will be asked to select between the two methods as shown below.

The screenshot shows the 'Digestion Times' screen with a blue background. At the top left, there is a green circular button with a white left arrow. The title 'Digestion Times' is in large white text, and 'Select Method' is in smaller white text below it. At the bottom, there are two green buttons: 'AOAC 991.43/ 985.29/2001.03' and 'AOAC 2009.01/ 2011.25'.

When you press the “AOAC 2009.01/2011.25” button the following screen will be displayed.



AOAC 2009.01/2011.25 Digestion Times
 (Press button with number to change)

Amylase/AMG Digestion Time (hours) 16.00 Save to Permanent Memory	Trizma Incubation Time (minutes) 20 SDF Precipitation Time (minutes) 60	Protease Digestion Time (minutes) 30 Reset to official method times
---	---	--

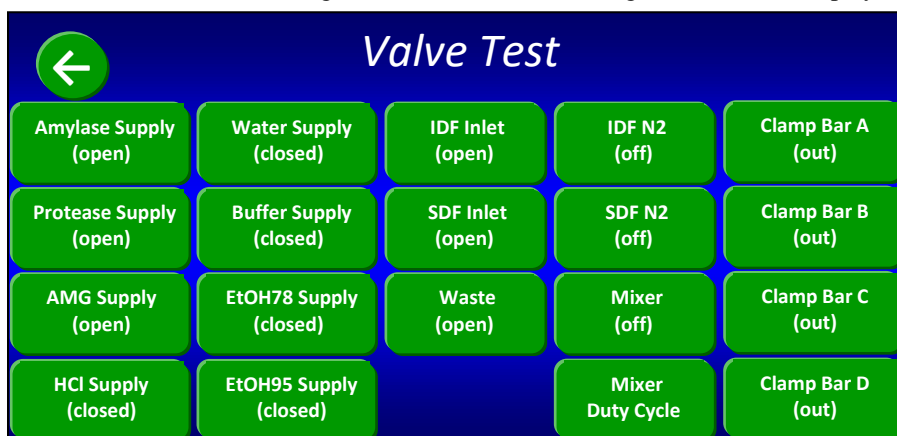
This diagnostic allows you to modify the times used by the computer for the digestion processes. This can be helpful when troubleshooting or demonstrating the instrument. If you press the “Save to Permanent Memory” button, the values you entered will be saved until you change them again by using this diagnostic. If you do not press the “Save to Permanent Memory” button, the values you entered will be saved until power is turned off, or until you change them again by using this diagnostic.

IMPORTANT:

If you start a new procedure with Digestion Times other than the official method times, a message will be displayed on the Touch Screen Display that allows you to reset the values. A Reset from this Warning Screen is only temporary. Use the Digestion Times screen in Diagnostics to make the reset permanent.

Mixer Duty Cycle

When you press the “Valve Test” button on the Diagnostics screen, the following screen will be displayed.



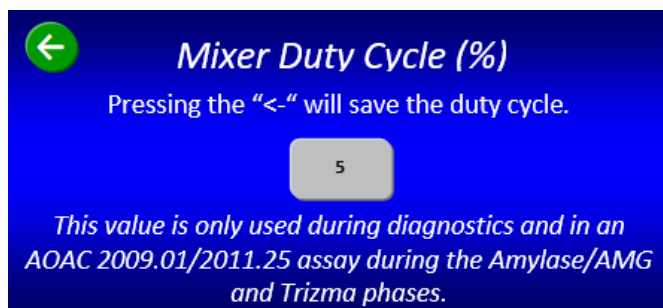
Valve Test

Amylase Supply (open)	Water Supply (closed)	IDF Inlet (open)	IDF N2 (off)	Clamp Bar A (out)
Protease Supply (open)	Buffer Supply (closed)	SDF Inlet (open)	SDF N2 (off)	Clamp Bar B (out)
AMG Supply (open)	EtOH78 Supply (closed)	Waste (open)	Mixer (off)	Clamp Bar C (out)
HCl Supply (closed)	EtOH95 Supply (closed)		Mixer Duty Cycle	Clamp Bar D (out)

When you press the “Mixer Duty Cycle” button on the Valve Test screen, the following screen will be displayed.

The number that you enter into the gray box on the above screen will be used during the Amylase/AMG digestion and Trizma phases. The default mixer duty cycle is 5%.

See the ANKOM^{TDF} Dietary Fiber Analyzer Operator’s Manual for more information about Diagnostics Mode.



Mixer Duty Cycle (%)
 Pressing the “<-” will save the duty cycle.

5

This value is only used during diagnostics and in an AOAC 2009.01/2011.25 assay during the Amylase/AMG and Trizma phases.

Appendix A – Reagents (AOAC 2009.01/2011.25)

Use Deionized (DI) or Distilled (DW) water throughout.

Best practices for solution preparation

The best practice for preparation of solutions is to make them fresh and use them within 2 days. This is especially true of the MES-TRIS buffer and Phosphate buffer solutions which can readily support microbial growth. Good laboratory cleaning practices are critical to ensure against contamination and microbial growth. Failure to maintain necessary cleanliness and prevent contamination can dramatically affect shelf life of solutions.

Reagents

- (a) *Ethanol 95%*.
- (b) *Ethanol 78%*—Place 821 ml 95% ethanol into 1 L volumetric flask and dilute to volume with water.
- (c) *Acetone*—reagent grade.
- (d) *Sodium maleate buffer solution*—50 mM, pH 6.0 and 2 mM CaCl₂. Dissolve 11.6 g of maleic acid in 1600 ml of water and adjust the pH to 6.0 with 4 M (160 g/L) NaOH solution. Add 0.6 g of calcium chloride (CaCl₂·2H₂O) and adjust the volume to 2 L.



Hazardous Materials – Only handle maleic acid after reviewing MSDS.

Do not add sodium azide to the buffer solution when using the TDF instrument. Although the methods call for sodium azide in the buffer as a preservative, it is not required for the method. The buffer solution should be made fresh each day. Strong reaction may occur if sodium azide is added to the buffer and used in the TDF instrument.

(e) *Enzyme Solutions*

The AOAC and AACC methods require the following enzyme activity per sample:

- Pancreatic α-Amylase: 2,000 Ceralpha Units
- Amyloglucosidase (AMG): 136 Glucose Units
- Protease: 35 Tyrosine Units

ANKOM ^{TDF} Enzyme Solutions	*Enzyme Concentration
<u>α-Amylase (Pancreatic)/AMG solution</u> 1. Measure 0.667 g α-Amylase Porcine Pancreatic (TDF130) into a 50 ml volumetric flask 2. Add 1.04 ml AMG concentrate (TDF84 or TDF85) to the same 50 ml volumetric flask 3. Make up to mark with sodium maleate buffer	1000 Ceralpha U/ml 68 Glucose U/ml
<u>Protease solution</u> 1. Dilute 5 ml Protease concentrate (TDF82 or TDF83) to 25 ml with DI or DW water	35 Tyrosine U/ml
*The ANKOM ^{TDF} Dietary Fiber Analyzer delivers 2 ml of the amylase/AMG solution and 1 ml of the protease solution to each of the six stations per run. If you are not using the ANKOM enzyme concentrates, you must prepare enzyme solutions to the recommended concentration, and it must include 0.02% w/v of Sodium Azide (to prevent microbial growth).	

- (f) *Diatomaceous earth (DE)*—(ANKOM DE1, DE2 or equivalent).
- (g) *Trizma Base, 0.75 M*—Add 90.8 g of Trizma (Sigma cat. No. T-1503) to ca 800 ml of water and dissolve. Adjust volume to 1 L.
- (h) *Acetic acid solution, 2 M*—Add 115 ml of glacial acetic acid (Fluka 45731) to a 1 L volumetric flask. Dilute to 1 L with water.
- (i) *D-Sorbitol (Internal standard solution)*—100 mg/ml. Weigh 100 g of dry D-Sorbitol into a beaker, dissolve in water and transfer to a 1 L volumetric flask with water and dilute to volume. Transfer to a polypropylene bottle and add 0.2 g of sodium azide as a preservative.

Appendix B - Analytical Procedures

IMPORTANT: While infrequent, procedures may be updated with new information. For the most up-to-date procedure revision refer to: <https://www.ankom.com/analytical-methods-support/tdf-analyzer>.

TDF Method (AOAC 2009.01, AACC 32-45.01) using the ANKOM^{TDF} Dietary Fiber Analyzer

Definition- Using Filter Bag Technology, this method determines the amount of TDF within a given sample using the weight of the recovered TDF residue corrected for ash and protein content.

Scope- Total, Soluble, and Insoluble Dietary Fiber in Foods and Feeds

Apparatus

1. Analytical Balance—capable of weighing 0.1 mg.
2. Oven—capable of maintaining a temperature of $105 \pm 2^\circ\text{C}$.
3. Fiber Recovery instrument capable of separately recovering IDF, SDF, and TDF residue. The instrument must be capable of automatically adding all reagents, mixing the sample to ensure proper digestion, and controlling digestion and precipitation temperatures (ANKOM^{TDF} Dietary Fiber Analyzer, ANKOM Technology).
4. Filter Bags (DF-I, DF-S, DF-FT, ANKOM Technology).
5. Bag Weigh Holder—used for eliminating static during bag weighing process (TDF52, ANKOM Technology).
6. Drying Rack—used for drying filter bags (TDF50, ANKOM Technology).
7. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure (HS, ANKOM Technology).
8. Desiccant Pouch—collapsible sealable pouch with desiccant inside that enables the removal of air from around the filter bags (X45, *Moisture Stop* weigh pouch, ANKOM Technology).
9. Marking pen—solvent and acid resistant (F06, ANKOM Technology).
10. Acetone rinse stand (TDF51 Rinse Stand, ANKOM Technology).

Reagents

Use Deionized (DI) or Distilled (DW) water throughout.

- (a) *Ethanol* 95%.
- (b) *Ethanol* 78%—Place 821 ml 95% ethanol into 1 L volumetric flask and dilute to volume with H₂O.
- (c) *Acetone*—reagent grade.
- (d) *Sodium maleate buffer solution*—50 mM, pH 6.0 and 2 mM CaCl₂. Dissolve 11.6 g of maleic acid in 1600 ml of water and adjust the pH to 6.0 with 4 M (160 g/L) NaOH solution. Add 0.6 g of calcium chloride (CaCl₂·2H₂O) and adjust the volume to 2 L.

IMPORTANT: Do not add sodium azide to the buffer solution when using the TDF instrument. Although the methods call for sodium azide in the buffer as a preservative, it is not required for the method. The buffer solution should be made fresh each day. Strong reaction may occur if sodium azide is added to the buffer and used in the TDF instrument.

(e) *Enzyme solutions*— The AOAC and AACC methods require the following enzyme activity per sample:

- Pancreatic α -Amylase: 2,000 Units
- Amyloglucosidase (AMG): 136 Units
- Protease: 35 Tyrosine Units

Prepare enzyme solutions for use in the ANKOM^{TDF} Dietary Fiber Analyzer as follows:

AOAC 2009.01/2011.01	Enzyme	Enzyme Concentration
	α -Amylase (Pancreatic)	2000 Ceralpha U/g sample
	Amyloglucosidase	136 Glucose U/g sample
	Protease	35 Tyrosine U/g sample
ANKOM ^{TDF} Enzyme Solutions		*Enzyme Concentration
<u>α-Amylase (Pancreatic)/AMG solution</u> 1. Measure 0.667 g α -Amylase Porcine Pancreatic (TDF130) into a 50 ml volumetric flask 2. Add 1.04 ml AMG concentrate (TDF84 or TDF85) to the same 50ml volumetric flask 3. Make up to mark with sodium maleate buffer		1000 Ceralpha U/ml 68 Glucose U/ml
<u>Protease solution</u> 1. Dilute 5 ml Protease concentrate (TDF82 or TDF83) to 25ml with DI or DW water		35 Tyrosine U/ml
*The ANKOM ^{TDF} Dietary Fiber Analyzer delivers 2 ml of the amylase/AMG solution and 1ml of the protease solution to each of the six stations per run. If you are not using the ANKOM enzyme concentrates, you must prepare enzyme solutions to the recommended concentration, and it must include 0.02% w/v of Sodium Azide (to prevent microbial growth).		

- (f) *Diatomaceous earth (DE)*—(ANKOM DE1/DE2, Celite 545 AW, No. C8656, Sigma Chemical Co. or equivalent).
- (g) *Trizma Base, 0.75 M*—Add 90.8 g of Trizma (Sigma cat. No. T-1503) to ca 800 ml of DI or DW water and dissolve. Adjust volume to 1 L.
- (h) *Acetic acid solution, 2 M*—Add 115 ml of glacial acetic acid (Fluka 45731) to a 1 L volumetric flask. Dilute to 1 L with DI or DW water.
- (i) *D-Sorbitol (Internal standard solution)*—100 mg/ml. Weigh 100 g of dry D-Sorbitol into a beaker, dissolve in water and transfer to a 1 L volumetric flask with water and dilute to volume. Transfer to a polypropylene bottle and add 0.2 g of Sodium Azide as a preservative. (NOTE: Handle sodium azide with caution only after reviewing MSDS.)

Calculations (all weights in grams)

$$\begin{aligned} \% \text{ TDF} &= \% \text{ WSWAIDF} + \% \text{ WASDF} \\ &= \left(\frac{\left[\frac{[(R_1 + R_2)/2] - P - A - B}{(M_1 + M_2)/2} \right] \times 100}{\left[\frac{[(f_{F1} - f_{S1} - D_1) + (f_{F2} - f_{S2} - D_2)]/2 - P - (A_2 - D_2) - B}{(M_1 + M_2)/2} \right] \times 100} \right) + \% \text{ WASDF} \end{aligned}$$

Where:

M_1, M_2	=	Original wt for duplicate samples adjusted for pre-treatment fat and sugar losses (g)
R_1, R_2	=	Residue for duplicate samples (g)
f_F	=	Final Filter Bag (g)
f_S	=	Initial Filter Bag (g)
D	=	Original wt of Diatomaceous Earth (g)
P	=	Protein of residue and bag (g)
A	=	Ash of residue and bag (g)
B	=	Blank (g)
	=	$[(BR_1 + BR_2)/2] - P_B - (A_B - D_B)$
	=	$[(f_{BF1} - f_{BS1} - D_{B1}) + (f_{BF2} - f_{BS2} - D_{B2})]/2 - P_{B1} - (A_{B2} - D_{B2})$
BR_1, BR_2	=	Residue for duplicate blanks (g)
f_{BF}	=	Final Blank Filter Bag (g)
f_{BS}	=	Initial Blank Filter Bag (g)
P_B	=	Protein of Blank Filter Bag (g)
A_B	=	Ash of Blank Filter Bag (g)
D_B	=	Original wt of Diatomaceous Earth in Blank Filter Bag (g)
WASDF	=	Soluble Dietary Fiber determined by HPLC

Sample Preparation

1. Grind samples in a centrifugal mill with a 0.5 mm screen. Samples ground finer may have particle loss from the filter bags and result in low values.
2. De-fat and de-sugar samples as needed based on the AOAC 2009.01 method and adjust sample weights accordingly.

TDF Procedure (see the TDF Analysis section of the Automated AOAC 2009.01/2011.25 and AACC 32-45.01 Method document for more details)

1. Label the filter bags using a solvent resistant marker.
2. Fill chemical containers to the Min. Level line or above.
3. Fill the Protease enzyme container to the 15 ml line or above. Fill the Amylase/AMG mixture and Trizma Base containers to the 30 ml line or above.
4. Place each filter bag in a tared Bag Weigh Holder and record the weight.
5. Place ca 1 g of DE in each of six tared and numbered tins and record the weights.
6. Place 0.5±0.05 g of sample in each of six tared and numbered tins and record the weights.
7. Remove all Clamp Bars from the instrument.
8. Follow the instructions on the Touch Screen Display (as detailed in steps 9-28 below).
9. Install SDF bags by gently pulling the black SDF Delivery Nozzle toward you and pulling each bag up so that the nozzle is inside the bag. Pull the bag up so that the top of the bag is about 35 mm (1.375 inches) above the top of Clamp Bar C and return the nozzle to its original position to hold the bag in place. Center each bag within the black lines located on the back of Clamp Bar C.
10. Re-install Clamp Bar D.
11. Flatten the bags to remove any wrinkles.
12. Press the check mark button (☑) on the "SDF Bags (and clamp bar D) installed?" screen on the Touch Screen Display. This will close bar D which will pinch the bags just above the filter.
13. Add DE to each SDF bag, rinsing the tin with no more than 3 ml of 78% to ensure complete transfer and that all the DE is below the SDF Delivery Nozzle.
14. Install IDF *Flow-Thru* bags by pulling the black IDF Delivery Nozzle toward you and pulling each bag up underneath the nozzle. Pull the bag up so that the top of the filter part of the IDF bag is just below the bottom of Clamp Bar B and return the nozzle to its original position to hold the bag in place. Center each bag within the black lines located on the back of Clamp Bar A.
15. Place at least 20 mm (0.75 inches) of the bottom of each IDF *Flow – Thru* bag inside the top of each corresponding SDF bag.
16. Re-install Clamp Bar B.
17. Flatten the bags to remove any wrinkles.
18. Press the check mark button (☑) on the "IDF Bags (and clamp bar B) installed?" screen on the Touch Screen Display. This will close bar B which will pinch the bags just above the filter.
19. Re-install Clamp Bar C.
20. Add sample into each of the IDF bags. Rinse the tin with no more than 3 ml of DI or DW water to ensure complete transfer and that all the sample is below the IDF Delivery Nozzle.
21. Secure the front of each SDF filter bag in place with the hook located on the front part of Clamp Bar C.

TDF Procedure (continued)

22. Check that the Nitrogen supply is connected to the instrument and turned on.
23. Set Filter Times.
24. Set the manual pH checks.
25. Add 1 ml of Internal Standard or instruct the instrument to prompt for a later addition.
26. Press the START button to begin the automated processes.
27. Check the pH after the Trizma Base has been added. (If configured, the instrument will stop so you can make this check.) Adjust to 7.9-8.4 as needed.
28. Check the pH after the Acetic Acid has been added. (If configured, the instrument will stop so you can make this check.) Adjust to approximately 4.3 as needed.
29. After the automated processes are complete, rinse the SDF bags twice with acetone. ANKOM recommends the use of the ANKOM TDF51 Rinse Stand for the acetone rinses.
30. After the acetone has evaporated, with your Heat Sealer set between 3 and 4 (settings may vary depending on the heat sealer and the power source), press the Heat Sealer arm down for 3 to 4 seconds to seal each bag just above the filter. This keeps all residue contained to the filter area while handling the bags.
31. Place each bag in the Drying Rack and place the rack in an oven set to 105°C. Dry to constant weight (about 90 min).
32. Remove all of the bags from the oven and place them in desiccant pouches to cool.
33. Removing only one filter bag from the desiccant pouches at a time, place each filter bag in a tared Bag Weigh Holder and record the weights.
34. Determine the protein content within the SDF residue. See the "Protein Determination Procedure – SDF / TDF" for more information.
35. Determine the ash content within the SDF residue. See the "Ash Determination Procedure – IDF / SDF / TDF" for more information.
36. Using an HPLC, determine the WASDF from the filtrate in the instrument Filtrate Cups.
37. Calculate % TDF values.

IDF/SDF Method (AOAC 2011.25, AACC 32-50.01) using the ANKOM^{TDF} Dietary Fiber Analyzer

Definition- Using Filter Bag Technology, this method determines the amount of IDF, SDF, and TDF within a given sample using the weight of the recovered IDF and SDF residue corrected for ash and protein content.

Scope- Total, Soluble, and Insoluble Dietary Fiber in Foods and Feeds

Apparatus

1. Analytical Balance—capable of weighing 0.1 mg.
2. Oven—capable of maintaining a temperature of $105 \pm 2^\circ\text{C}$.
3. Fiber Recovery instrument capable of separately recovering IDF, SDF, and TDF residue. The instrument must be capable of automatically adding all reagents, mixing the sample to ensure proper digestion, and controlling digestion and precipitation temperatures (ANKOM^{TDF} Dietary Fiber Analyzer, ANKOM Technology).
4. Filter Bags (DF-I, DF-S, DF-FT, ANKOM Technology).
5. Bag Weigh Holder—used for eliminating static during bag weighing process (TDF52, ANKOM Technology).
6. Drying Rack—used for drying filter bags (TDF50, ANKOM Technology).
7. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure (HS, ANKOM Technology).
8. Desiccant Pouch—collapsible sealable pouch with desiccant inside that enables the removal of air from around the filter bags (X45, Moisture Stop weigh pouch, ANKOM Technology).
9. Marking pen—solvent and acid resistant (F06, ANKOM Technology).
10. Acetone rinse stand (TDF51 Rinse Stand, ANKOM Technology).

Reagents

Use Deionized water (DI) or Distilled (DW) throughout.

- (a) *Ethanol* 95%.
- (b) *Ethanol* 78%—Place 821 ml 95% ethanol into 1 L volumetric flask and dilute to volume with H₂O.
- (c) *Acetone*—reagent grade.
- (d) *Sodium maleate buffer solution*—50 mM, pH 6.0 and 2 mM CaCl₂. Dissolve 11.6 g of maleic acid in 1600 ml of water and adjust the pH to 6.0 with 4 M (160 g/L) NaOH solution. Add 0.6 g of calcium chloride (CaCl₂·2H₂O). Adjust the volume to 2 L.

IMPORTANT: Do not add sodium azide to the buffer solution when using the TDF instrument. Although the methods call for sodium azide in the buffer as a preservative, it is not required for the method. The buffer solution should be made fresh each day. Strong reaction may occur if sodium azide is added to the buffer and used in the TDF instrument.

- (e) *Enzyme solutions*— The AOAC and AACC methods require the following enzyme activity per sample:

- Pancreatic α -Amylase: 2,000 Units
- Amyloglucosidase (AMG): 136 Units
- Protease: 35 Tyrosine Units

Prepare enzyme solutions for use in the ANKOM^{TDF} Dietary Fiber Analyzer as follows:

AOAC 2009.01/2011.01	Enzyme	Enzyme Concentration
	α -Amylase (Pancreatic)	2000 Ceralpha U/g sample
	Amyloglucosidase	136 Glucose U/g sample
	Protease	35 Tyrosine U/g sample
ANKOM ^{TDF} Enzyme Solutions		*Enzyme Concentration
<u>α-Amylase (Pancreatic)/AMG solution</u> 1. Measure 0.667 g α -Amylase Porcine Pancreatic (TDF130) into a 50 ml volumetric flask 2. Add 1.04 ml AMG concentrate (TDF84 or TDF85) to the same 50 ml volumetric flask 3. Make up to mark with sodium maleate buffer		1000 Ceralpha U/ml 68 Glucose U/ml
<u>Protease solution</u> 1. Dilute 5 ml Protease concentrate (TDF82 or TDF83) to 25ml with DI or DW water		35 Tyrosine U/ml
*The ANKOM ^{TDF} Dietary Fiber Analyzer delivers 2 ml of the amylase/AMG solution and 1ml of the protease solution to each of the six stations per run. If you are not using the ANKOM enzyme concentrates, you must prepare enzyme solutions to the recommended concentration, and it must include 0.02% w/v of Sodium Azide (to prevent microbial growth).		

- (f) *Diatomaceous earth (DE)*—(ANKOM DE1/DE2, Celite 545 AW, No. C8656, Sigma Chemical Co. or equivalent).
- (g) *Trizma Base, 0.75 M*—Add 90.8 g of Trizma (Sigma cat. No. T-1503) to ca 800 ml of DI or DW water and dissolve. Adjust volume to 1 L.
- (h) *Acetic acid solution, 2 M*—Add 115 ml of glacial acetic acid (Fluka 45731) to a 1 L volumetric flask. Dilute to 1 L with DI or DW water.
- (i) *D-Sorbitol (Internal standard solution)*—100 mg/ml. Weigh 100 g of dry D-Sorbitol into a beaker, dissolve in water and transfer to a 1 L volumetric flask with water and dilute to volume. Transfer to a polypropylene bottle and add 0.2 g of Sodium Azide as a preservative. (NOTE: Handle sodium azide with caution only after reviewing MSDS.)

Calculations (all weights in grams)

$$\begin{aligned} \% \text{ IDF} &= \left(\frac{[(R_1 + R_2)/2] - P - A - B}{(M_1 + M_2)/2} \right) \times 100 \\ &= \left(\frac{[(f_{F1} - f_{S1}) + (f_{F2} - f_{S2})]/2 - P - A - B}{(M_1 + M_2)/2} \right) \times 100 \end{aligned}$$

Where:

M_1, M_2	=	Original wt for duplicate samples adjusted for pre-treatment fat and sugar losses (g)
R_1, R_2	=	Residue for duplicate samples (g)
f_F	=	Final Filter Bag (g)
f_S	=	Initial Filter Bag (g)
P	=	Protein of residue and bag (g)
A	=	Ash of residue and bag (g)
B	=	Blank (g)
	=	$[(BR_1 + BR_2)/2] - P_B - A_B$
	=	$[(f_{BF1} - f_{BS1}) + (f_{BF2} - f_{BS2})]/2 - P_B - A_B$
BR_1, BR_2	=	Residue for duplicate blanks (g)
f_{BF}	=	Final Blank Filter Bag (g)
f_{BS}	=	Initial Blank Filter Bag (g)
P_B	=	Protein of Blank Filter Bag (g)
A_B	=	Ash of Blank Filter Bag (g)

$$\begin{aligned} \% \text{ SDF} &= \% \text{ WSWAIDF} + \% \text{ WASDF} \\ &= \left(\left(\frac{[(R_1 + R_2)/2] - P - A - B}{(M_1 + M_2)/2} \right) \times 100 \right) + \% \text{ WASDF} \\ &= \left(\left(\frac{[(f_{F1} - f_{S1} - D_1) + (f_{F2} - f_{S2} - D_2)]/2 - P - (A_2 - D_2) - B}{(M_1 + M_2)/2} \right) \times 100 \right) + \% \text{ WASDF} \end{aligned}$$

Where:

M_1, M_2	=	Original wt for duplicate samples adjusted for pre-treatment fat and sugar losses (g)
R_1, R_2	=	Residue for duplicate samples (g)
f_F	=	Final Filter Bag (g)
f_S	=	Initial Filter Bag (g)
D	=	Original wt of Diatomaceous Earth (g)
P	=	Protein of residue and bag (g)
A	=	Ash of residue and bag (g)
B	=	Blank (g)
	=	$[(BR_1 + BR_2)/2] - P_B - (A_B - D_B)$
	=	$[(f_{BF1} - f_{BS1} - D_{B1}) + (f_{BF2} - f_{BS2} - D_{B2})]/2 - P_{B1} - (A_{B2} - D_{B2})$
BR_1, BR_2	=	Residue for duplicate blanks (g)
f_{BF}	=	Final Blank Filter Bag (g)
f_{BS}	=	Initial Blank Filter Bag (g)
P_B	=	Protein of Blank Filter Bag (g)
A_B	=	Ash of Blank Filter Bag (g)
D_B	=	Original wt of Diatomaceous Earth in Blank Filter Bag (g)
WASDF	=	Soluble Dietary Fiber determined by HPLC

$$\% \text{ TDF} = \% \text{ IDF} + \% \text{ SDF}$$

Sample Preparation

1. Grind samples in a centrifugal mill with a 0.5 mm screen. Samples ground finer may have particle loss from the filter bags and result in low values.
2. De-fat and de-sugar samples as needed based on the AOAC 2011.25 method and adjust sample weights accordingly.

IDF/SDF Procedure (see the IDF/SDF Analysis section of the Automated AOAC 2009.01/2011.25 and AACC 32-45.01 Method document for more details)

1. Label the filter bags using a solvent resistant marker.
2. Fill chemical containers to the Min. Level line or above.
3. Fill the Protease enzyme container to the 15 ml line or above. Fill the Amylase/AMG mixture and Trizma Base containers to the 30 ml line or above.
4. Place each filter bag in a tared Bag Weigh Holder and record the weight.
5. Place ca 1 g of DE in each of six tared and numbered tins and record the weights.
6. Place 0.5±0.05 g of sample in each of six tared and numbered tins and record the weights.
7. Remove all Clamp Bars from the instrument.
8. Follow the instructions on the Touch Screen Display (as detailed in steps 9-28 below).
9. Install SDF bags by gently pulling the black SDF Delivery Nozzle toward you and pulling each bag up so that the nozzle is inside the bag. Pull the bag up so that the top of the bag is about 35 mm (1.375 inches) above the top of Clamp Bar C and return the nozzle to its original position to hold the bag in place. Center each bag within the black lines located on the back of Clamp Bar C.
10. Re-install Clamp Bar D.
11. Flatten the bags to remove any wrinkles.
12. Press the check mark button (☑) on the "SDF Bags (and clamp bar D) installed?" screen on the Touch Screen Display. This will close bar D which will pinch the bags just above the filter.
13. Add DE to each SDF bag, rinsing the tin with no more than 3 ml of 78% to ensure complete transfer and that all the DE is below the SDF Delivery Nozzle.
14. Install IDF bags by gently pulling the black IDF Delivery Nozzle toward you and pulling each bag up underneath the nozzle. Pull the bag up so that the top of the filter part of the IDF bag is just below the bottom of Clamp Bar B and return the nozzle to its original position to hold the bag in place. Center each bag within the black lines located on the back of Clamp Bar A.
15. Place at least 20 mm (0.75 inches) of each IDF bag filter inside the top of each corresponding SDF bag.
16. Re-install Clamp Bar B.
17. Flatten the bags to remove any wrinkles.
18. Press the check mark button (☑) on the "IDF Bags (and clamp bar B) installed?" screen on the Touch Screen Display. This will close bar B which will pinch the bags just above the filter.
19. Re-install Clamp Bar C.
20. Add sample into each of the IDF bags. Rinse the tin with no more than 3 ml of DI or DW water to ensure complete transfer and that all the sample is below the IDF Delivery Nozzle.
21. Secure the front of each SDF filter bag in place with the hook located on the front part of Clamp Bar C.
22. Check that the Nitrogen supply is connected to the instrument and turned on.
23. Set Filter Times.
24. Set the manual pH checks.
25. Add 1 ml of Internal Standard or instruct the instrument to prompt for a later addition.
26. Press the START button to begin the automated processes.

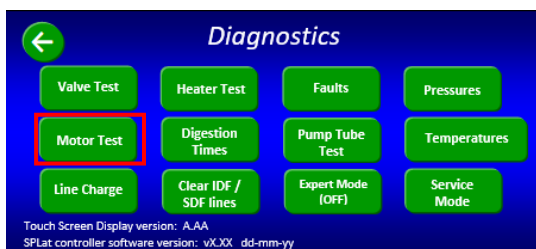
IDF/SDF Procedure (continued)

27. Check the pH after the Trizma Base has been added. (If configured, the instrument will stop so you can make this check.) Adjust to 7.9-8.4 as needed.
28. Check the pH after the Acetic Acid has been added. (If configured, the instrument will stop so you can make this check.) Adjust to approximately 4.3 as needed.
29. After the automated processes are complete, rinse the IDF and SDF bags twice with acetone. ANKOM recommends the use of the ANKOM TDF51 Rinse Stand for the acetone rinses.
30. After the acetone has evaporated, with your Heat Sealer set between 3 and 4 (settings may vary depending on the heat sealer and the power source), press the Heat Sealer arm down for 3 to 4 seconds to seal each bag just above the filter. This keeps all residue contained to the filter area while handling the bags.
31. Place each bag in the Drying Rack and place the rack in an oven set to 105°C. Dry to constant weight (about 90 min).
32. Remove all of the bags from the oven and place them in desiccant pouches to cool.
33. Removing only one filter bag from the desiccant pouches at a time, place each filter bag in a tared Bag Weigh Holder and record the weights.
34. Determine the protein content within the IDF residue. See the "Protein Determination Procedure – IDF" for more information.
35. Determine the protein content within the SDF residue. See the "Protein Determination Procedure – SDF / TDF" for more information.
36. Determine the ash content within the IDF and SDF residue. See the "Ash Determination Procedure – IDF / SDF / TDF" for more information.
37. Using an HPLC, determine the WASDF from the filtrate in the instrument Filtrate Cups.
38. Calculate the % IDF and % SDF values.
39. Calculate % TDF by adding the % IDF and % SDF values.

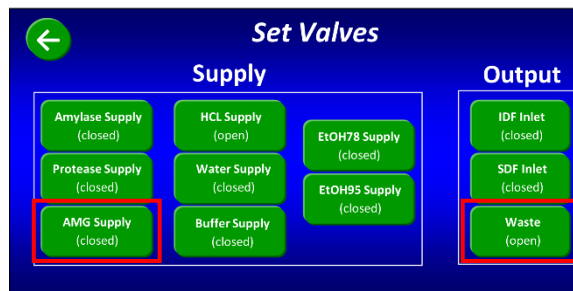
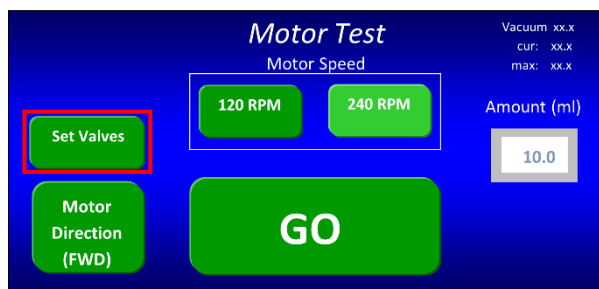
Appendix C- TRIZMA Line Flush

Crystallization of TRIZMA Base in the silicone tubing can happen overnight and will cause a fault during a run the next day. To prevent this, it is necessary to flush this line out at the end of each day that the instrument has been used.

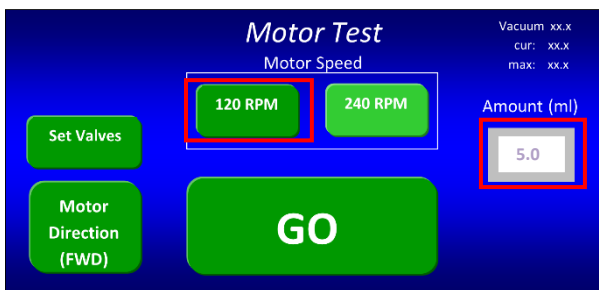
1. Fill an enzyme vial with 30 ml of Deionized (DI) or Distilled (DW) water.
2. Connect it to the TRIZMA (AMG) fitting on the enzyme container bracket.
3. Go to “Diagnostics” and press “Motor Test.”



4. Select “Set Valves”, open the “AMG Supply” Valve and the “Waste” output valve. Close all other valves.



5. Press the back arrow to return to the main “Motor Test” screen.
6. Set the volume to 5 ml.
7. Set speed to 120 RPM.



8. Press “GO” (30 ml will empty the vial).

This page intentionally left blank

Automation saves time and money!

ANKOM Technology is an international company with products that include...



FLEX Analyte Extractor

- Vitamin Analysis - Automatic saponification, extraction, and evaporation of solvent in one instrument
- Eliminates bi-phase extractions
- State-of-the-art software allows method adjustment and customization



DELTA Fiber Analyzer

- Crude Fiber (AOCS Ba 6a-05), ADF, NDF
- An internal pump system allows the user to connect to and draw from any chemical source within range of the instrument, eliminating the need to lift heavy cubetainers.
- Batch process up to 24 samples at one time



XT15 Fat Extractor

- Official Method AOCS Am 5-04
- Fully automatic
- Solvent recovery at 97% or greater
- Batch process - up to 15 samples at one time



RF Gas Production System

- High sensitivity pressure measurement
- Applications include: Biomass-to-Energy analysis (e.g., Ethanol, methane, etc.), Biodegradability, Ruminant Nutrition, Yeast Activity, Beer/Wine Fermentation, Soil respiration, BOD, Human Digestion, etc.
- Wireless Computer control and data storage



Chemicals

- A wide variety of chemicals used for many different lab operations
- Pre-mixed solutions available

Please visit our web site at www.ankom.com for more information.

2052 O'Neil Rd, Macedon NY 14502
Telephone: (315) 986-8090
Fax: (315) 986-8091
www.ankom.com

ANKOM
TECHNOLOGY