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Introduction

ANKOM Technology designs, manufactures, and markets instruments and support products used by analytical laboratories around the world in the environmental, agricultural, biomass, and food industries. ANKOM Technology can provide you with products for determining or monitoring detergent fibers, dietary fibers, fat, digestibility, microbial fermentation (anaerobic or aerobic) and more.

Committed to Total Customer Satisfaction, ANKOM designs every product based on a thorough assessment of customer needs.

Congratulations on your purchase of the ANKOM²⁰⁰⁰ Fiber Analyzer. We are confident that this product will effectively serve your needs.

By carefully following the operating instructions in this manual, you will minimize errors in results. Experience indicates that errors in results are usually associated with minor variations in carrying out the procedure. This manual will provide you with details that will help assure accuracy of your results.

NOTE:

Please review the entire contents of this manual before you begin operating this instrument.

Warranty

ANKOM Technology warrants the ANKOM²⁰⁰⁰ Fiber Analyzer against any defects due to faulty workmanship or material for one year after the original date of purchase. This warranty does not include damage to the instrument resulting from neglect or misuse. During the warranty period, should any failure result from defects in workmanship or materials, ANKOM Technology will, at its discretion, repair or replace the instrument free of charge.

Extended warranties are available for purchase if desired.

Filter Bags

ANKOM Technology filter bags (part # F57 and F58) are designed to produce precision and accurate fiber results. The F57 bags should be used when grinding samples using a cutter type mill with a 1 mm screen which is typically referenced by the approved methods. For finer ground samples or if particle loss is suspected, use the F58 bags which have a finer pore size.

Use of other types of filtration media not tested and approved by ANKOM Technology may cause damage to electrical valves and other components and void your warranty. Filter bags can be purchased from ANKOM Technology or from your local authorized ANKOM distributor.

Operating Environment

Your ANKOM²⁰⁰⁰ Fiber Analyzer is designed to operate within the following environments:

- Ambient Temperature Range: 15°–30°C
- Humidity: 20–60% RH
- Power (domestic): 110V–120V ~ 50/60Hz 15A
- Power (international): 220V–240V ~ 50/60Hz 10A

Contact Information

At ANKOM Technology we are committed to your total satisfaction and therefore always available to help you get the most from your ANKOM products. We are also very interested in any comments or suggestions you may have to help us improve.

For any questions or suggestions regarding your instrument, please contact us at:

- Telephone: (315) 986-8090
- Fax: (315) 986-8091
- Email: service@ankom.com
- **www.ankom.com**

Instrument Description

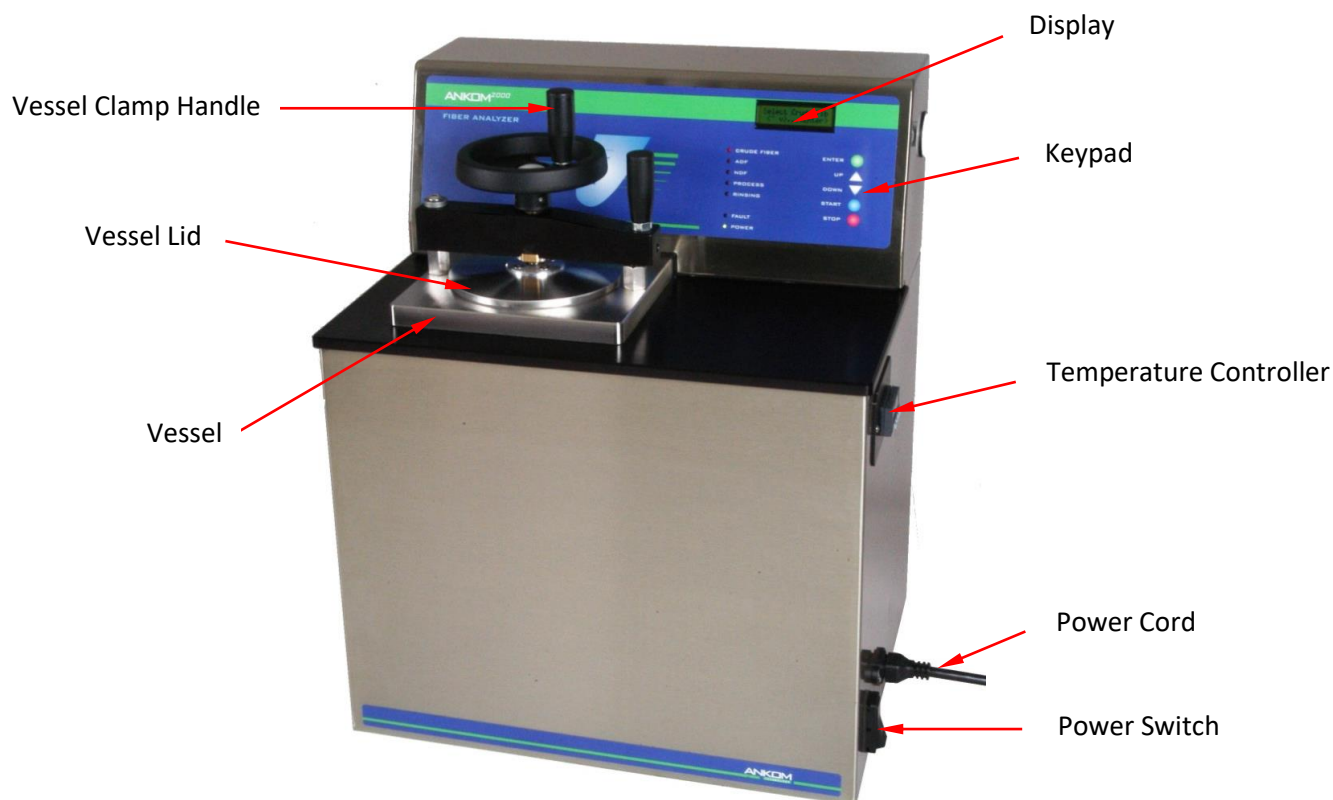
General Description

The ANKOM²⁰⁰⁰ Fiber Analyzer is designed to efficiently and accurately determine Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), and Crude Fiber within food and/or feed samples. Enabled by Filter Bag Technology, up to 24 samples can be processed at one time.

During analysis cell contents are removed as the encapsulated sample is subjected to the appropriate chemical (AD, ND, or crude fiber acid and base) solutions, leaving the desired fiber fraction. Results are determined gravimetrically. The filter bags are designed to allow proper flow of solutions while retaining non-soluble components. The fiber residue captured in the filter bag can be used for follow-on assays such as ADIN, NDIN, and ADL.

Like the ANKOM²⁰⁰ Fiber Analyzer, digestion and rinse operations are all performed within the same instrument, allowing for the elimination of the separate filtration step. Process temperatures are precisely controlled while providing proper agitation to ensure a uniform flow of chemical solutions and rinses across each sample.

Below are detailed views of the ANKOM²⁰⁰⁰ Fiber Analyzer.



Port A – Used for Crude Fiber
Acid solution and ND solution

Vent Tube

Water Supply Fitting

Water Filter

Line to Hot Water Supply

Drain Hose



Left Side View

Port B – Used for Crude Fiber Base solution, AD
solution, and Diluted Amylase

Temperature Controller

Fuses

Domestic:	15A/120V
International:	10A/220V

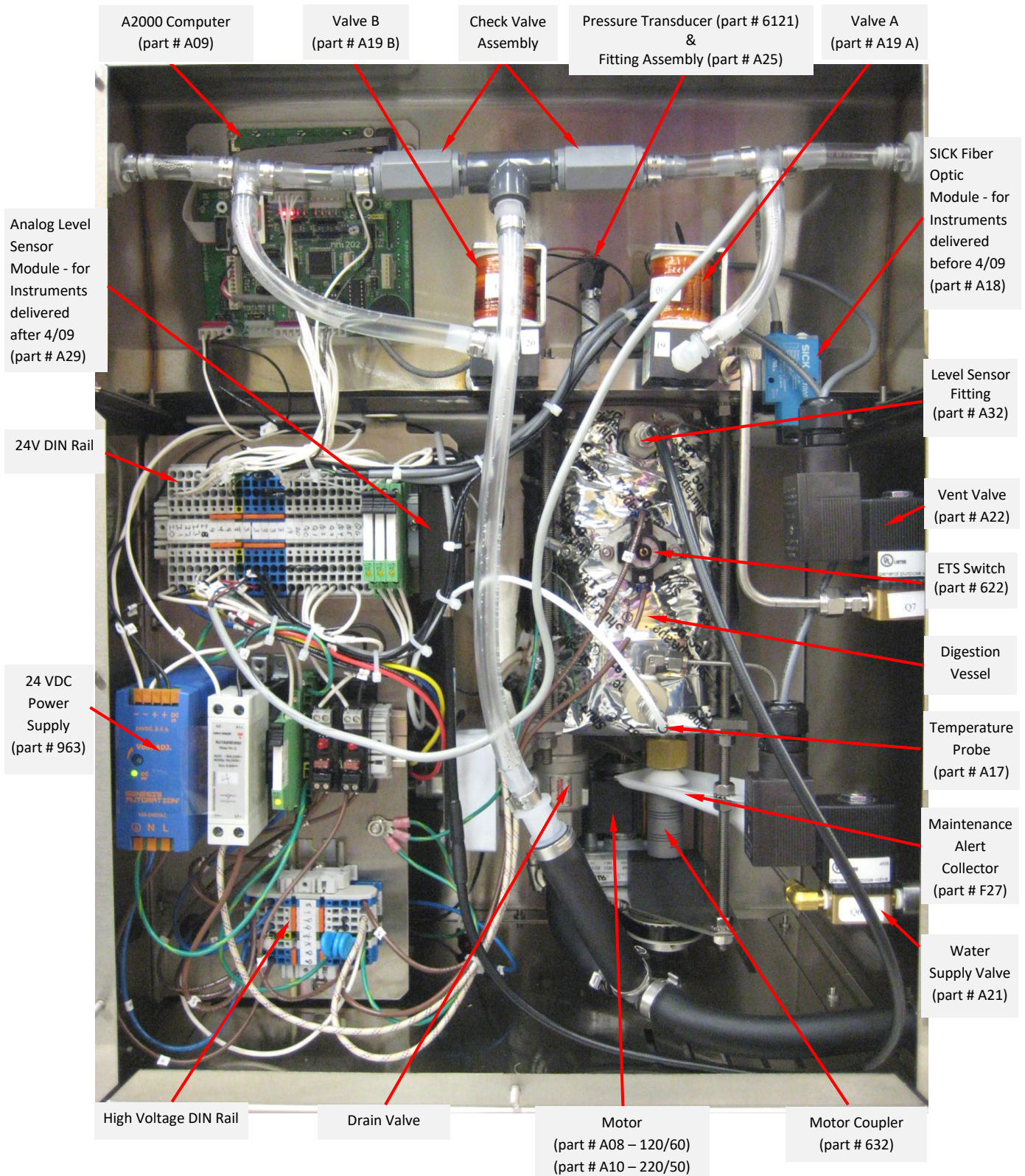
Power Cord Inlet

Power Switch



Right Side View

Internal Components



Safety Precautions



Hazardous Pressure – Do NOT open the Vessel Lid during operation. The contents of the Vessel are hot and under pressure. **Failure to observe this caution may result in scalding or burning.**

Hot Surfaces – Do NOT touch the Vessel surfaces during operation. The surface can exceed 70°C (158°F). **Failure to observe this caution may result in burning.**

Hazardous Voltages – Do NOT operate the instrument with the cover removed. Hazardous voltages are present during operation. The Power Cord must be disconnected prior to removal of the rear panel. **Failure to observe this caution may result in electrical shock or electrocution.**

Hazardous Materials – Caution should be used when handling hot effluent that may be caustic or corrosive. If necessary, the solution can be collected in a container and neutralized before disposal. Follow safe laboratory practices according to your local regulations when installing and using this instrument and associated chemicals.

WARNING: Attempts to override safety features or to use this instrument in a manner not specified by ANKOM Technology voids the warranty and may result in serious injury or even death.

This system is designed to meet and/or exceed the applicable standards of CE, CSA, NRTL and OSHA.

IMPORTANT:

- The Power Switch must be in the OFF position before plugging the instrument Power Cord into the power source.
- In the event of an instrument malfunction, the internal heater will be automatically turned off by one of the following safety devices:
 - 1) Electrical Fuses
 - 2) The Emergency Temperature Shut-off Switch (ETS)
 - 3) The Pressure Transducer
- Do NOT open the Vessel Lid during or after an operation until both pressure and liquid are thoroughly exhausted. Connect and secure the Drain Hose along the path to the drain so it will not move when hot pressurized fluid is exhausted. **Failure to secure the hose could result in uncontrolled chemical flow.**

NOTE:

Please review the entire contents of this manual before you begin operating this instrument.

Instrument Installation

Site Requirements

To install and operate the ANKOM²⁰⁰⁰ Fiber Analyzer you will need the following:

- Adjustable wrench
- Water supply located within 6 feet (3 meters) of the ANKOM²⁰⁰⁰ and capable of heating water to 50°C for Crude Fiber and 70°C for ADF/NDF analyses
- Adequate power (see “Operating Environment” section)
- Drain

Instrument Installation Procedure

To install the ANKOM²⁰⁰⁰ Fiber Analyzer, follow the procedure detailed below.

1. Remove the instrument from the shipping container and place it in an area that is within 6 feet of a drain and water supply on a surface that is firm and level. The instrument must not be subject to excessive shock, vibration, dirt, moisture, oil, or other fluids.

IMPORTANT: Do NOT place this instrument near microwave ovens or mechanical devices.

Your instrument comes complete with a Power Cord, a Drain Hose, a Vent Tube, a Bag Suspender Assembly (including Bag Suspender Trays and a Bag Suspender Weight), and an Amylase Container.



2. With the Power Switch in the OFF position, plug the Power Cord into the Power Cord Inlet on the instrument.
3. Plug the Power Cord into the power source.
4. Install the Water Filter assembly. Attach ¼” copper tubing to the hot water source (+50°C Crude Fiber, +70°C ND and AD) and to the Water Supply Fitting located on the left side of the instrument.
5. Connect and secure the Drain Hose so that it will not move when hot pressurized fluid is exhausted.

6. If you are using Cubitainers for your chemicals, install the Cubitainer Shelf shown below (Optional).


IMPORTANT:

The solutions are gravity fed into the instrument ports. The bottom of the solution level must be at least 5" (13cm) above the port. The top of the solution level can be no more than 20" (50cm) above either port or the solution will overflow into the drain.

Fiber Analysis Support Items

The following support items are needed to perform the fiber analysis procedures:

Item	Recommended Product
Electronic Balance with four-place readout	ANKOM #TB Balance Hardware ANKOM #TBS Balance Software
Filter Bags	ANKOM #F57, #F58
Bag Holder (used for adding sample to an empty filter bag)	ANKOM #101.2
Heat Sealer for sealing the filter bags	ANKOM #1915 (120V), #1920 (220V)
Solvent Resistant Marker	ANKOM #F08
Desiccant Pouch	ANKOM #X45
Oven for drying (capable of maintaining 102°C ± 2°)	ANKOM #RD (120V), #RDI (220V)
Sample	
Spoon	

Analysis Options using the ANKOM²⁰⁰⁰ Fiber Analyzer

The ANKOM²⁰⁰⁰ Fiber Analyzer can be configured to run ADF, NDF, and Crude Fiber analyses. The instrument will run with default digestion and rinse time settings unless you select the Custom analysis option. This option allows you to do ADF, NDF, or Crude Fiber analyses using custom digestion and rinse time settings.

For maintenance purposes, the ANKOM²⁰⁰⁰ also provides the capability to flush the solution lines.

The following sections provide the information you will need to use and maintain the ANKOM²⁰⁰⁰ Fiber Analyzer.

ADF Analysis

ADF Calculation

ADF contained within a food or feed sample can be calculated using the following formula:

% ADF (as-received basis)	=	$\frac{100 \times (W_3 - (W_1 \times C_1))}{W_2}$
Where:		
	W_1	= Bag tare weight
	W_2	= Sample weight
	W_3	= Dried weight of filter bag with fiber after extraction process
	C_1	= Blank bag correction (running average of final oven-dried weight divided by original blank bag weight)

ADF Sample Preparation Procedure

To prepare samples for fiber analysis, follow the procedure detailed below.

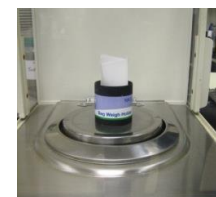
IMPORTANT:

When using the ANKOM²⁰⁰⁰ Fiber Analyzer for ADF analysis, at least one blank filter bag should be included with the sample set as an indicator of particle loss. A running average of the blank bag weights is used in the fiber calculation as the C_1 correction factor. A C_1 value larger than 1.0000 indicates that sample particles were lost from filter bags and deposited on the blank bag. Any fiber particle loss from the filter bags will generate erroneous results. If particle loss is observed, the grinding method for the specific sample should be evaluated.

1. Using a Solvent Resistant Marker, number all of the filter bags you will use during the fiber analysis. Allow the marker to dry on the filter bags.
2. Weigh and record the weight of each empty filter bag (W_1).
3. Set the Heat Sealer dial to between 4 and 5. (The setting may vary from sealer to sealer.)
4. Seal at least one empty filter bag (to be used as a blank) within 4mm of its open end. Keep the sealer arm down for 2 – 3 seconds after the red sealer light turns off (to cool the seal). The seal can be seen as a solid melted stripe along the top edge of the filter bag (as shown to the right). If the seal is not strong, re-seal the bag.
5. Place an empty filter bag in the Bag Holder in an open position.
6. Tare the weight of the empty filter bag and the holder together.
7. Add 0.45 – 0.50g of sample to the filter bag. Keep all particles away from the sealing area of the filter bag.
8. Record the weight of the sample (W_2) and tare.



Seal



9. Seal the filter bag within 4mm of its open end. Keep the sealer arm down for 2 – 3 seconds after the red sealer light turns off (to cool the seal). The seal can be seen as a solid melted stripe along the top edge of the filter bag. If the seal is not strong, re-seal the bag.
10. Spread the sample out uniformly within the filter bag by shaking and flicking the bag to eliminate clumping.
11. Repeat steps 5 – 10 for all filter bags that will be used in the Analyzer. (Up to 24 bags can be processed during one procedure.)
12. Follow these pre-extraction steps for specified samples.

12.1 If your samples contain non-roasted soybean or >5% fat:

Before doing the ADF analysis in the ANKOM^{DELTA}, you will need to do a pre-extraction. For samples containing non-roasted soybean or >5% fat, follow the pre-extraction steps below:

- 12.1.1 Place the filter bags with sample (up to 23) into a container with a top.
- 12.1.2 Pour enough fresh acetone into the container to cover the bags.
- 12.1.3 Put the top on the container.
- 12.1.4 Allow bags to soak for 10 minutes.
- 12.1.5 Pour out and dispose of the acetone.
- 12.1.6 Execute steps 1 through 5 a total of two times.
- 12.1.7 Place the bags on a wire screen to air-dry.

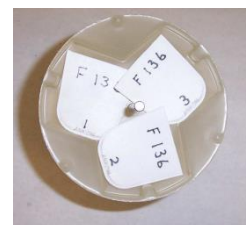
12.2 If your samples contain roasted soybean:

Before doing the ADF analysis in the ANKOM^{DELTA}, you will need to do a pre-extraction. For samples containing roasted soybean, follow the pre-extraction steps below:

- 12.2.1 Place the filter bags with sample (up to 23) into a container with a top.
- 12.2.2 Put the top on the container.
- 12.2.3 Shake the container 10 times.
- 12.2.4 Allow the samples to soak for twelve hours.
- 12.2.5 Pour out the acetone

Place the bags on a wire screen to air-dry.

13. If the sample has settled to the bottom of the bag, spread the sample uniformly inside the filter bags by shaking and flicking the bags to eliminate clumping.
14. Place the filter bags with sample and at least one empty bag (used as a blank) into the Bag Suspender trays as shown (maximum of three bags per tray).
15. Stack each tray on the Bag Suspender rod (eight trays in total) with each tray rotated 120 degrees from the tray below.



IMPORTANT: You must use all eight trays even if they are empty.

16. Add the 9th tray to the top of the Bag Suspender rod. This tray contains no filter bags and acts as a cover.

NOTE: The samples are now ready for the ADF analysis procedure.

ADF Analysis Procedure using the ANKOM²⁰⁰⁰ Fiber Analyzer

NOTE:

If you are following the acid detergent procedure with an acid detergent lignin, you can find the lignin procedure on our website at www.ankom.com.

To perform ADF analysis on prepared samples, follow the procedure detailed below.

IMPORTANT:

If you are changing from one analysis type to another, flush the system before running. See the "Flush Procedure" section of this manual for more information.

1. Verify that the hot water supply is on and the drain hose is securely positioned in the drain.
2. If you are using Cubitainers for your chemicals, attach the AD solution hose first to the Cubitainer and then to Port B on the instrument.



IMPORTANT:

The solutions are gravity fed into the instrument ports. The bottom of the solution level must be at least 5" (13cm) above the port. The top of the solution level can be no more than 20" (50cm) above either port or the solution will overflow into the drain.

3. Open the Vessel Lid.
4. Place the Bag Suspender with the samples into the Vessel.
5. Place the Bag Suspender Weight onto the Bag Suspender rod to hold the trays in place.
6. Turn the instrument Power Switch to the ON position. The Display will light and allow you to select an analysis procedure.
7. Press the arrow keys on the Keypad until you see "Select ADF" on the Display.

Select ADF
^ v <Enter>

IMPORTANT:

The ADF analysis will use the following default settings:

- 60 minute digestion
- four 5 minute rinses

If you want to run a custom ADF analysis that allows you to set the digestion time and the number of rinses, press the arrow keys until you see "Select Custom" on the Display.

8. Press ENTER on the Keypad and follow the prompts on the Display to set up the instrument for ADF analysis.
9. Close the Vessel Lid and tighten it by turning the Vessel Clamp Handle.

**NOTE:**

If you do not want to use the Cubitainers to automatically add your solutions, you can manually fill the vessel for each procedure. First make sure that the hoses to the Cubitainers are disconnected. Immediately after executing step 10 below, pour 2L of solution directly into the vessel. Please note that the solution must cover the level sensor in order for the instrument to start its operation. Once agitation begins, close the Vessel Lid and continue the procedure with step 11.

10. Press START on the Keypad. Solution from the Cubitainer will flow into the vessel through Port B.

NOTE:

Once the analysis begins, digestions and rinses occur automatically, with the analyzer Display providing information about the process time remaining, the temperature, and the pressure. Pressing STOP on the Keypad at any time during the analysis ends the operation and opens the drain to exhaust the solution.

11. When the “Extraction Done” message appears on the Display, the analyzer operation is complete. Open the Vessel Lid, remove the bags and place them in an appropriately sized beaker.
12. With your hands, gently press out excess water from the bags into the beaker and pour off the water from the beaker.
13. With the bags in the beaker, add enough acetone to cover them. Let the bags soak in acetone for 3 – 5 minutes. Then pour off the acetone.
14. With your hands, gently press out excess acetone from the bags into the beaker and pour off the acetone from the beaker.
15. Remove the bags from the beaker and place them on a wire screen to air-dry.



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

16. Place air-dried bags in the oven and heat at $102^{\circ}\text{C} \pm 2^{\circ}$ for 2 – 4 hours (depending on the oven).

IMPORTANT:

When running a lignin procedure or a sequential (NDF/ADF or NDF/ADF/Lignin) with F57 Filter Bags it is important not to dry the bags overnight after the NDF or ADF procedure. A drying timeframe of 2-4 hours at 100°C to 105°C is sufficient to thoroughly dry the bags after each procedure. Extended drying times or too high a temperature can compromise the bag's filtration media. In addition, after the fourth rinse during the ADF procedure if you would like to confirm the acid has been completely rinsed from the bags, capture some water from the waste tube and check the water with litmus paper.

17. Remove the bags from the oven and place them in a Desiccant Pouch.



IMPORTANT: Do NOT use conventional countertop or cabinet desiccators for this analysis.

18. Allow the bags to cool to room temperature. This should take about 10 – 15 minutes.
19. Remove one filter bag from the Desiccant Pouch. Press the pouch to remove ambient air and zip it tight.
20. Re-weigh the filter bag (W_3) immediately.
21. Repeat steps 19 and 20 for each filter bag in the Desiccant Pouch.
22. Calculate ADF using the following formula.

$$\% \text{ ADF (as-received basis)} = \frac{100 \times (W_3 - (W_1 \times C_1))}{W_2}$$

Where:

W_1	=	Bag tare weight
W_2	=	Sample weight
W_3	=	Dried weight of filter bag with fiber after extraction process
C_1	=	Blank bag correction (running average of final oven-dried weight divided by original blank bag weight)

NOTE: If you are following the acid detergent procedure with an acid detergent lignin, you can find the lignin procedure on our website at www.ankom.com.

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NDF Analysis

NDF Calculation

NDF contained within a food or feed sample can be calculated using the following formula:

% NDF (as-received basis)	=	$\frac{100 \times (W_3 - (W_1 \times C_1))}{W_2}$
Where:		
	W_1	= Bag tare weight
	W_2	= Sample weight
	W_3	= Dried weight of filter bag with fiber after extraction process
	C_1	= Blank bag correction (running average of final oven-dried weight divided by original blank bag weight)

NDF Sample Preparation Procedure

To prepare samples for fiber analysis, follow the procedure detailed below.

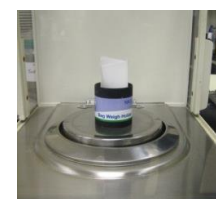
IMPORTANT:

When using the ANKOM²⁰⁰⁰ Fiber Analyzer for NDF analysis, at least one blank filter bag should be included with the sample set as an indicator of particle loss. A running average of the blank bag weights is used in the fiber calculation as the C_1 correction factor. A C_1 value larger than 1.0000 indicates that sample particles were lost from filter bags and deposited on the blank bag. Any fiber particle loss from the filter bags will generate erroneous results. If particle loss is observed, the grinding method for the specific sample should be evaluated.

1. Using a Solvent Resistant Marker, number all of the filter bags you will use during the fiber analysis. Allow the marker to dry on the filter bags.
2. Weigh and record the weight of each empty filter bag (W_1).
3. Set the Heat Sealer dial to between 4 and 5. (The setting may vary from sealer to sealer.)
4. Seal at least one empty filter bag (to be used as a blank) within 4mm of its open end. Keep the sealer arm down for 2 – 3 seconds after the red sealer light turns off (to cool the seal). The seal can be seen as a solid melted stripe along the top edge of the filter bag (as shown to the right). If the seal is not strong, re-seal the bag.
5. Place an empty filter bag in the Bag Holder in an open position.
6. Tare the weight of the empty filter bag and the holder together.
7. Add 0.45 – 0.50g of sample to the filter bag. Keep all particles away from the sealing area of the filter bag.
8. Record the weight of the sample (W_2) and tare.



Seal



9. Seal the filter bag within 4mm of its open end. Keep the sealer arm down for 2 – 3 seconds after the red sealer light turns off (to cool the seal). The seal can be seen as a solid melted stripe along the top edge of the filter bag. If the seal is not strong, re-seal the bag.
10. Spread the sample out uniformly within the filter bag by shaking and flicking the bag to eliminate clumping.
11. Repeat steps 5 – 10 for all filter bags that will be used in the Analyzer. (Up to 24 bags can be processed during one procedure.)
12. Follow these pre-extraction steps for specified samples.

12.1 If your samples contain non-roasted soybean or >5% fat:

Before doing the ADF analysis in the ANKOM^{DELTA}, you will need to do a pre-extraction. For samples containing non-roasted soybean or >5% fat, follow the pre-extraction steps below:

- 12.1.1 Place the filter bags with sample (up to 23) into a container with a top.
- 12.1.2 Pour enough fresh acetone into the container to cover the bags.
- 12.1.3 Put the top on the container.
- 12.1.4 Allow bags to soak for 10 minutes.
- 12.1.5 Pour out and dispose of the acetone.
- 12.1.6 Execute steps 1 through 5 a total of two times.
- 12.1.7 Place the bags on a wire screen to air-dry.

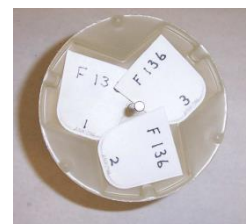
12.2 If your samples contain roasted soybean:

Before doing the ADF analysis in the ANKOM^{DELTA}, you will need to do a pre-extraction. For samples containing roasted soybean, follow the pre-extraction steps below:

- 12.2.1 Place the filter bags with sample (up to 23) into a container with a top.
- 12.2.2 Put the top on the container.
- 12.2.3 Shake the container 10 times.
- 12.2.4 Allow the samples to soak for twelve hours.
- 12.2.5 Pour out the acetone

Place the bags on a wire screen to air-dry.

13. If the sample has settled to the bottom of the bag, spread the sample uniformly inside the filter bags by shaking and flicking the bags to eliminate clumping.
14. Place the filter bags with sample and at least one empty bag (used as a blank) into the Bag Suspender trays as shown (maximum of three bags per tray).
15. Stack each tray on the Bag Suspender rod (eight trays in total) with each tray rotated 120 degrees from the tray below.



IMPORTANT: You must use all eight trays even if they are empty.

16. Add the 9th tray to the top of the Bag Suspender rod. This tray contains no filter bags and acts as a cover.

NOTE: The samples are now ready for the NDF analysis procedure.

NDF Analysis Procedure using the ANKOM²⁰⁰⁰ Fiber Analyzer

To perform NDF analysis on prepared samples, follow the procedure detailed below.

IMPORTANT:

- If you are changing from one analytical method to another, flush the system before running. See the "Flush Procedure" section of this manual for more information.
- Amylase tends to be sticky. You should always clean the Amylase Dispenser Assembly and flush Port B after every NDF procedure unless you are running another NDF procedure with less than two hours between procedures.

1. Verify that the hot water supply is on and the drain hose is securely positioned in the drain.
2. If you are using Cubitainers for your chemicals, attach the ND solution hose first to the Cubitainer and then to Port A on the instrument.

**IMPORTANT:**

The solutions are gravity fed into the instrument ports. The bottom of the solution level must be at least 5" (13cm) above the port. The top of the solution level can be no more than 20" (50cm) above either port or the solution will overflow into the drain.

3. Open the Vessel Lid.
4. Place the Bag Suspender with the samples into the Vessel.
5. Place the Bag Suspender Weight onto the Bag Suspender rod to hold the trays in place.
6. Attach the Amylase Dispenser Assembly to Port B. This will be used to automatically add amylase to the vessel during the rinses.
7. Add water until the dispenser is 25% full.
8. Add two capfuls (8ml) of amylase to the dispenser.
9. Add water until the dispenser is 75% full.
10. Turn the instrument Power Switch to the ON position. The Display will light and allow you to select an analysis procedure.



11. Press the arrow keys on the Keypad until you see “Select NDF” on the Display.

Select NDF
^ v <Enter>

IMPORTANT: The NDF analysis will use the following default settings:

- 75 minute digestion
- four 5 minute rinses

If you want to run a custom NDF analysis that allows you to set the digestion time and the number of rinses, press the arrow keys until you see “Select Custom” on the Display.

12. Press ENTER on the Keypad and follow the prompts on the Display to set up the instrument for NDF analysis.

NOTE:

If you do not want to use the Cubitainers to automatically add your solutions, you can manually fill the vessel for each procedure. First make sure that the hoses to the Cubitainers are disconnected. Immediately after executing step 13 below, pour 2L of solution directly into the vessel. Please note that the solution must cover the level sensor in order for the instrument to start its operation. Continue the procedure with step 14.

13. Press START on the Keypad. Solution from the Cubitainer will flow into the vessel through Port A.

NOTE:

Once the analysis begins, digestions and rinses occur automatically, with the analyzer Display providing information about the process time remaining, the temperature, and the pressure. Pressing STOP on the Keypad at any time during the analysis ends the operation and opens the drain to exhaust the solution.

14. After the ND solution has been added and agitation begins, manually add 20g of Na_2SO_3 and 4.0ml of alpha-amylase directly into the Vessel.
15. Close the Vessel Lid and tighten it by turning the Vessel Clamp Handle.



16. When the “Extraction Done” message appears on the Display, the analyzer operation is complete. Open the Vessel Lid, remove the bags and place them in an appropriately sized beaker.
17. With your hands, gently press out excess water from the bags into the beaker and pour off the water from the beaker.
18. With the bags in the beaker, add enough acetone to cover them. Let the bags soak in acetone for 3 – 5 minutes. Then pour off the acetone.
19. With your hands, gently press out excess acetone from the bags into the beaker and pour off the acetone from the beaker.



20. Remove the bags from the beaker and place them on a wire screen to air-dry.



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

21. Place air-dried bags in the oven and heat at $102^{\circ}\text{C} \pm 2^{\circ}$ for 2 – 4 hours (depending on the oven).

IMPORTANT: When running a lignin procedure or a sequential (NDF/ADF or NDF/ADF/Lignin) with F57 Filter Bags it is important not to dry the bags overnight after the NDF or ADF procedure. A drying timeframe of 2-4 hours at 100°C to 105°C is sufficient to thoroughly dry the bags after each procedure. Extended drying times or too high a temperature can compromise the bag's filtration media. In addition, after the fourth rinse during the ADF procedure if you would like to confirm the acid has been completely rinsed from the bags, capture some water from the waste tube and check the water with litmus paper.

22. Remove the bags from the oven and place them in a Desiccant Pouch.



IMPORTANT: Do NOT use conventional countertop or cabinet desiccators for this analysis.

23. Allow the bags to cool to room temperature. This should take about 10 – 15 minutes.
24. Remove one filter bag from the Desiccant Pouch. Press the pouch to remove ambient air and zip it tight.
25. Re-weigh the filter bag (W_3) immediately.
26. Repeat steps 24 and 25 for each filter bag in the Desiccant Pouch.
27. Calculate NDF using the following formula.

$\% \text{ NDF (as-received basis)} = \frac{100 \times (W_3 - (W_1 \times C_1))}{W_2}$	
Where:	<p>W_1 = Bag tare weight</p> <p>W_2 = Sample weight</p> <p>W_3 = Dried weight of filter bag with fiber after extraction process</p> <p>C_1 = Blank bag correction (running average of final oven-dried weight divided by original blank bag weight)</p>

IMPORTANT: Amylase is sticky. Unless you are running multiple NDF procedures in near succession, you must flush the system after NDF procedures. See the "Flush Procedure" section of this manual for details.

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Crude Fiber Analysis

Crude Fiber Calculation

Crude Fiber contained within a food or feed sample can be calculated using the following formula:

% Crude Fiber	=	$\frac{100 \times (W_3 - (W_1 \times C_1))}{W_2}$
Where:		
W ₁	=	Bag tare weight
W ₂	=	Sample weight
W ₃	=	Weight of Organic Matter (loss of weight on ignition of bag and fiber)
C ₁	=	Ash corrected blank bag factor (running average of loss of weight on ignition of blank bag / original blank bag)

Crude Fiber Sample Preparation Procedure

To prepare samples for fiber analysis, follow the procedure detailed below.

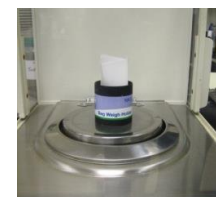
IMPORTANT:

When using the ANKOM²⁰⁰⁰ Fiber Analyzer for Crude Fiber analysis, at least one blank filter bag should be included with the sample set as an indicator of particle loss. A running average of the blank bag weights is used in the fiber calculation as the C₁ correction factor. A C₁ value larger than 1.0000 indicates that sample particles were lost from filter bags and deposited on the blank bag. Any fiber particle loss from the filter bags will generate erroneous results. If particle loss is observed, the grinding method for the specific sample should be evaluated.

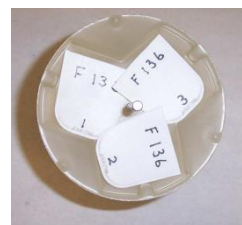
1. Using a Solvent Resistant Marker, number all of the filter bags you will use during the fiber analysis. Allow the marker to dry on the filter bags.
2. Weigh and record the weight of each empty filter bag (W₁).
3. Set the Heat Sealer dial to between 4 and 5. (The setting may vary from sealer to sealer.)
4. Seal at least one empty filter bag (to be used as a blank) within 4mm of its open end. Keep the sealer arm down for 2 – 3 seconds after the red sealer light turns off (to cool the seal). The seal can be seen as a solid melted stripe along the top edge of the filter bag (as shown to the right). If the seal is not strong, re-seal the bag.
5. Place an empty filter bag in the Bag Holder in an open position.
6. Tare the weight of the empty filter bag and the holder together.
7. Add 0.95 – 1.00g of sample to the filter bag. Keep all particles away from the sealing area of the filter bag.
8. Record the weight of the sample (W₂) and tare.



Seal



9. Seal the filter bag within 4mm of its open end. Keep the sealer arm down for 2 – 3 seconds after the red sealer light turns off (to cool the seal). The seal can be seen as a solid melted stripe along the top edge of the filter bag. If the seal is not strong, re-seal the bag.
10. Spread the sample out uniformly within the filter bag by shaking and flicking the bag to eliminate clumping.
11. Repeat steps 5 – 10 for all filter bags that will be used in the Analyzer. (Up to 24 bags can be processed during one procedure.)
12. **For all samples you will need to do a pre-extraction of fat.**
Before doing a Crude Fiber analysis in the ANKOM^{DELTA}, follow the pre-extraction steps below:
 - 12.1 Place the filter bags with sample into an appropriately sized container.
 - 12.2 Place the filter bags with sample into an appropriately sized container.
 - 12.3 Pour enough petroleum ether into the container to cover the bags.
 - 12.4 Allow the bags to soak for 10 minutes.
 - 12.5 Pour out and dispose of the petroleum ether.
 - 12.6 Place the bags on a wire screen to air-dry.
13. If the sample has settled to the bottom of the bag, spread the sample uniformly inside the filter bags by shaking and flicking the bags to eliminate clumping.
14. Place the filter bags with sample and at least one empty bag (used as a blank) into the Bag Suspender trays as shown (maximum of three bags per tray).
15. Stack each tray on the Bag Suspender rod (eight trays in total) with each tray rotated 120 degrees from the tray below.



IMPORTANT: You must use all eight trays even if they are empty.

16. Add the 9th tray to the top of the Bag Suspender rod. This tray contains no filter bags and acts as a cover.

NOTE: The samples are now ready for the Crude Fiber analysis procedure.

Crude Fiber Analysis Procedure using the ANKOM²⁰⁰⁰ Fiber Analyzer

To perform Crude Fiber analysis on prepared samples, follow the procedure detailed below.

IMPORTANT: If you are changing from one analysis type to another, flush the system before running. See the "Flush Procedure" section of this manual for more information.

1. Verify that the hot water supply is on and the drain hose is securely positioned in the drain.
2. Attach each Cubitainer hose first to the Cubitainer and then to its specific chemical port. Port A is used for Crude Fiber Acid solution. Port B is used for Crude Fiber Base solution.



IMPORTANT: The solutions are gravity fed into the instrument ports. The bottom of the solution level must be at least 5" (13cm) above the port. The top of the solution level can be no more than 20" (50cm) above either port or the solution will overflow into the drain.

3. Open the Vessel Lid.
4. Place the Bag Suspender with the samples into the Vessel.
5. Place the Bag Suspender Weight onto the Bag Suspender rod to hold the trays in place.
6. Turn the instrument's Power Switch to the ON position. The Display will light and allow you to select an analysis procedure.
7. Press the arrow keys on the Keypad until you see "Select Crude Fib" on the Display.

Select Crude Fib
^ v <Enter>

IMPORTANT: The Crude Fiber analysis will use the following default settings:

- 40 minute Acid digestion
- 40 minute Base digestion
- two 5 minute Acid rinses
- three 5 minute Base rinses

If you want to run a custom Crude Fiber analysis that allows you to set the digestion times and the number of rinse cycles, press the arrow keys until you see "Select Custom" on the Display.

8. Press ENTER on the Keypad and follow the prompts on the Display to set up the instrument for Crude Fiber analysis.

9. Close the Vessel Lid tightening it by turning the Vessel Clamp Handle.

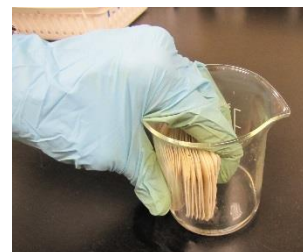


10. Press START on the Keypad. Solution will flow first into the vessel through Port A.

NOTE:

Once the analysis begins, digestions and rinses occur automatically, with the analyzer Display providing information about the process time remaining, the temperature, and the pressure. Pressing STOP on the Keypad at any time during the analysis ends the operation and opens the drain to exhaust the solution.

11. When the “Extraction Done” message appears on the Display, the analyzer operation is complete. Open the Vessel Lid, remove the bags and place them in an appropriately sized beaker.
12. With your hands, gently press out excess water from the bags into the beaker and pour off the water from the beaker.
13. With the bags in the beaker, add enough acetone to cover them. Let the bags soak in acetone for 3 – 5 minutes. Then pour off the acetone.
14. With your hands, gently press out excess acetone from the bags into the beaker and pour off the acetone from the beaker.
15. Remove the bags from the beaker and place them on a wire screen to air-dry.



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

16. Place air-dried bags in the oven and heat at $102^{\circ}\text{C} \pm 2^{\circ}$ for 2 – 4 hours (depending on the oven).
17. Remove the bags from the oven and place them in a Desiccant Pouch.

IMPORTANT: Do NOT use conventional countertop or cabinet desiccators for this analysis.



18. Allow the bags to cool to room temperature. This should take about 10 – 15 minutes.
19. Re-weigh each filter bag immediately after removing from the Desiccant Pouch.
20. Ash all filter bags in pre-weighed crucibles for 2 hours at $600^{\circ}\text{C} \pm 15^{\circ}$.
21. Cool the ashed crucibles in a conventional desiccator.
22. Weigh the ashed crucibles to calculate the loss of weight of organic matter (W_3).
23. Calculate Crude Fiber using the following formula.

% Crude Fiber	=	$\frac{100 \times (W_3 - (W_1 \times C_1))}{W_2}$
Where:		
W_1	=	Bag tare weight
W_2	=	Sample weight
W_3	=	Weight of Organic Matter (loss of weight on ignition of bag and fiber)
C_1	=	Ash corrected blank bag factor (running average of loss of weight on ignition of blank bag / original blank bag)

Flush Procedure

The Flush procedure allows you to clean the system with water. This procedure should be used when changing from one procedure to another (e.g., when preparing to do an ADF analysis after completing an NDF analysis) or when doing an NDF procedure after a previous NDF procedure with more than two hours between procedures, or before storing the instrument.

IMPORTANT:

Amylase tends to be sticky. You should always clean the Amylase Dispenser Assembly and flush Port B after every NDF procedure unless the next procedure you are running is another NDF, and you run it within two hours of the previous procedure.

To perform a Flush of your analyzer, follow the instructions below.

1. Turn the instrument's Power Switch to the ON position. The Display will light.
2. Verify that the water supply is on and the drain hose is securely in the drain.
3. Press the arrow keys on the Keypad until you see "Select Flush" on the Display.
4. Press ENTER on the Keypad.
5. Attach the Amylase Dispenser Assembly to port B.
6. Fill the dispenser with about 100 ml of hot water.
7. Attach the Amylase Dispenser Assembly to port A.
8. Fill the dispenser with about 100 ml of hot water.

Select Flush
^ v <Enter>

**NOTE:**

If you press and hold the START key on the Keypad during the Flush operation, water will flow into the Vessel. This will rinse the bottom of the Vessel, but it will not rinse all the way to the top. If you need to rinse anything from the top part of the inside of the Vessel, pour hot water into the Vessel as needed during the Flush operation.

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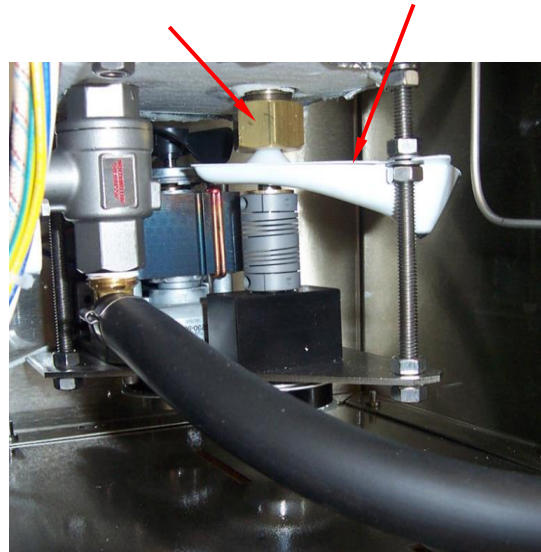
Periodic Maintenance

Initial Maintenance after the first 10 hours of operation, and then periodic maintenance after every 80 hours of operation.

After the first 10 hours of operation, follow the procedure below:

1. Remove the back panel of the instrument.
2. Inspect the Maintenance Alert Collector.
3. Clean any residue from the collector.
4. Unscrew the filter body (the fixture on the outside of the instrument that attaches to the water supply).
5. Unscrew and clean the metal filter screen.
6. Reassemble the filter and compression fitting. Make sure the valve body is tight and the washer has not come out of the filter body.

Packing Nut Maintenance Alert Collector



Rear View of instrument with cabinet back removed

IMPORTANT: Do NOT insert the Bag Suspender into the instrument for this procedure.

7. Press the arrow keys on the Keypad until you see "Select Flush" on the Display. Press "ENTER" on the Keypad to turn the agitator on.

Select Flush
^ v <Enter>

8. When the motor is activated, turn the Packing Nut to the **RIGHT** until you hear a change in the sound of the motor. (The motor will start to labor as the packing nut gets harder to turn.)
9. Loosen the Packing Nut slightly until the motor stops laboring.
10. Turn off the instrument and re-install the back panel.



Repeat the Maintenance procedure (steps 1 – 10 above) after every 80 hours of operation.

If you see a leak

If you see a leak, execute the Initial Maintenance procedure above, loosening the Packing Nut 1/16 of a turn. If the

leak continues, replace the packing and agitator.

Replace the Fuses (as needed)

To replace the fuses in the ANKOM²⁰⁰⁰ Fiber Analyzer, follow the procedure detailed below.

1. Turn off the instrument power and unplug the Power Cord from the outlet.
2. Using a flat blade screwdriver, twist the slot on the fuse holders counterclockwise ¼ turn to open.
3. Check both fuses.
4. Pull the fuse from the grey fuse cap and replace as needed.

(120V – 15 amp / 220V – 10 amp)

Fuses (2)



Clean the Fiber Optic Level Sensor

Using a cotton swab with alcohol, wipe the tip of the level sensor at least once per month if you use the instrument daily.

Level Sensor



Check the Agitation System

The agitation system should be checked every three to six months or if fiber values are higher than normal or inconsistent. To check the agitation system, follow the steps below.

IMPORTANT: Poor agitation will cause higher analysis values and poor repeatability.

1. Check the function of the agitator motor.

- a. Place a bag suspender with all 9 trays in the vessel along with the bag suspender weight, but add NO water.
- b. Turn the instrument's Power Switch to the ON position. The Display will light.
- c. Press the UP/DOWN arrow buttons on the Keypad until you see "Select Flush" on the Display.
- d. Press ENTER on the Keypad to turn the agitator on.
- e. Verify that the bag suspender moves up 16 times in 15 seconds (65 rpm).
- f. Press ENTER on the Keypad to turn the agitator off.



2. Mark the agitator stroke.

- a. Leaving the bag suspender in the vessel, remove the bag suspender weight.
- b. Remove the top from a dark felt tip marker and lay the marker horizontally on the top of the bag suspender so that the tip touches the inside wall of the vessel.
- c. With constant, light, downward pressure on the marker, hold the pen in place so that it rides the top tray up and down once the agitation has begun.
- d. With "Select Flush" on the Display, press ENTER on the Keypad to begin agitation.
- e. Allow the bag suspender (& pen) to move up and down three or four times as the pen marks the vessel wall.
- f. Press ENTER on the Keypad to stop the agitation.



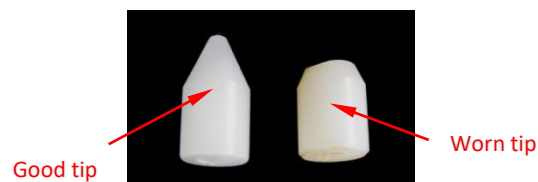
3. Measure the agitator stroke.

- a. Turn off the power switch.
- b. Remove the pen and the bag suspender from the vessel.
- c. Measure the mark on the vessel wall. It should be 1/2 inch in length.



4. Replace a worn Kynar Tip (if necessary).

If the motion is less than 1/2 inch, you will need to replace either the Bag Suspender Tip (Kynar Tip w/ washer Assembly - part # 11.5) or the agitator (Agitator Assembly - part # 8.9) because the old disc has flattened.



Check the Temperature Control using the Boil Test

To check the ANKOM²⁰⁰⁰ Fiber Analyzer temperature control, do a Boil Test *once per month* as specified in the procedure below.

1. Disconnect cubitainers if connected.
2. Fill a pitcher with approximately 2L of hot water (50 °C or above) and set aside for later.
3. Turn on the instrument. Remove the bag suspender from the vessel.

4. When you see

Select Crude Fib
v <Enter>

on the display, press the ENTER button.

5. When you see

Insert Samples
Close Lid<Enter>

on the display, press the ENTER button.

(Do not insert samples. Do not close lid.)

6. When you see

Hot Water On
<Start>

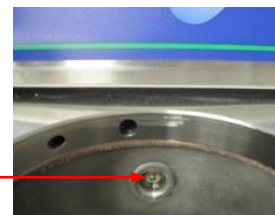
on the display, press the START button.

7. Record Voltage:

- a. Record the voltage shown on the display before pouring in the water. _____V
(Should be between 5-10 VDC)
- b. Pour the 2L of hot water into the digestion vessel so that the glass level sensor tip is under water.
- c. Record the voltage after pouring in the water. _____V
(Should be between 0-3 VDC)

Agitation and heat begin automatically.

Level Sensor Tip



8. Do not close the lid. Occasionally stir the water to ensure uniform heating.
The water should begin to boil in 5-15 minutes.

9. When a strong boil is reached, stir the water in the vessel and record:

- a. The temperature on the controller. _____°C
- b. The temperature on a calibrated thermometer. _____°C
(Place the thermometer near the probe at the bottom of the vessel.)
- c. The temperature shown on the display. _____°C

Controller



Calibrated
Thermometer

Temperature Probe



IMPORTANT:

If these three temperatures are not within ½ degree of each other, contact ANKOM Technology for assistance. Send results to service@ankom.com.

Check the Bag Suspender

The bag suspender should be checked every three to six months or if fiber values are higher than normal or inconsistent. To check the Bag Suspender, follow the steps below.

1. **Verify that you are using the NEW version of the Bag Suspender Trays.**

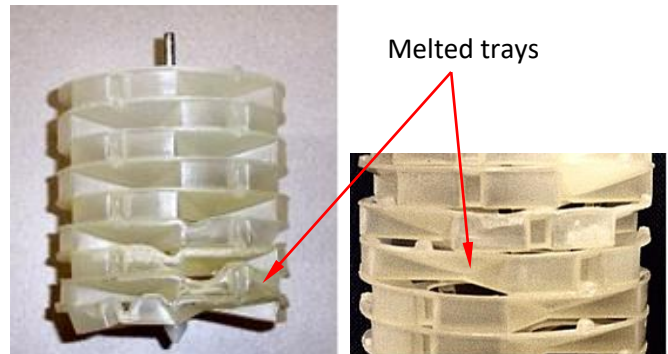
The NEW version of the trays has 9 holes in groups of 3 as shown in the picture to the right.



IMPORTANT: You CANNOT use the new and old bag suspender trays together.

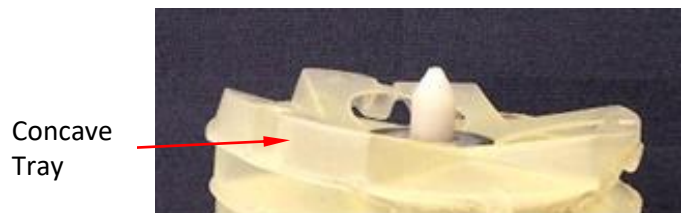
2. **Check the trays for melting.**

The pictures shown are examples of extreme cases. However, for proper operation you must replace trays that show signs of melting or wear.



3. **Check that the bottom tray is flat.**

If the bottom tray is concave (see picture) the bag suspender will catch in the vessel and melt.



Troubleshooting & Replacement Parts

The ANKOM Technology web site has the most current troubleshooting and replacement parts information. Therefore, if you have any questions about the operation of your ANKOM²⁰⁰⁰ Fiber Analyzer, or if you need replacement parts, please visit our web site at www.ankom.com.

Appendix A - 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/fiber-analyzer-a2000>.

Acid Detergent Fiber in Feeds - Filter Bag Technique (for A2000 and A2000I)

Definition: This method determines Acid Detergent Fiber, which is the residue remaining after digesting with H₂SO₄ and CTAB. The fiber residues are predominantly cellulose and lignin.

Scope: This method is applicable to grains, feeds, forages, and all fiber-bearing material.

Apparatus

1. Analytical Balance—capable of weighing 0.1 mg.
2. Oven—capable of maintaining a temperature of $102 \pm 2^\circ\text{C}$.
3. Digestion instrument—capable of performing the digestion at $100 \pm 0.5^\circ\text{C}$ and maintaining a pressure of 10-25psi. The instrument must be capable of creating a similar flow around each sample to ensure uniformity of extraction (ANKOM²⁰⁰⁰ with 65rpm agitation, ANKOM Technology).
4. Filter Bags—constructed from chemically inert and heat resistant filter media, capable of being heat sealed closed and able to retain 25-micron particles while permitting solution penetration (F57 and F58, ANKOM Technology).
5. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure (HS or HSi, ANKOM Technology).
6. Desiccant Pouch—collapsible sealable pouch with desiccant inside that enables the removal of air from around the filter bags (MoistureStop weigh pouch, ANKOM Technology).
7. Marking pen—solvent and acid resistant (F08, ANKOM Technology).

Reagents

1. Acid Detergent Solution—Add 20g cetyl trimethylammonium bromide (CTAB) to 1L 1.00N H₂SO₄ previously standardized (premixed chemical solution available from ANKOM). Agitate and heat to aid solution.
CAUTION1: Sulfuric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. Always add acid to water and not the reverse.
CAUTION2: CTAB will irritate mucous membranes. A dust mask and gloves should be worn when handling this chemical.

Sample Preparation

Grind samples in a centrifugal mill with a 2mm screen or cutter type (Wiley) mill with a 1mm screen. Samples ground finer may have particle loss from the filter bags and result in low values.

ADF Procedure (see the ADF Analysis section of the Operator's Manual for more detail)

1. Use a solvent resistant marker to label the filter bags to be used in the analysis.
2. Weigh and record the weight of each empty filter bag (W_1) and zero the balance. NOTE: Do not pre-dry filter bags. Any moisture will be accounted for by the blank bag correction.
3. Place 0.45 – 0.50g of prepared sample in up to 23 of the bags and record the weight (W_2) of each. Avoid placing the sample in the upper 4mm of the bag.
4. Include at least one empty bag in the run to determine the blank bag correction (C_1).
 NOTE: A running average blank bag correction factor (C_1) should be used in the calculation of fiber. The inclusion of at least one blank bag in each run is mainly used as an indicator of particle loss. A C_1 larger than 1.0000 indicates that sample particles were lost from filter bags and deposited on the blank bag during the extraction. Any fiber particle loss from the filter bags will generate erroneous results. If particle loss is observed, then the grinding method needs to be evaluated.
5. Using a heat sealer, completely seal each filter bag closed within 4mm of the top to encapsulate the sample. NOTE: Use sufficient heat to completely seal the filter bags and allow enough cool time (2 sec) before removing each bag from the heat sealer.
6. Spread the sample uniformly inside the filter bags by shaking and flicking the bags to eliminate clumping.
7. **Pre-extract only samples containing >5% fat:** Extract samples by placing bags with samples into a container with a top. Pour enough acetone into the container to cover the bags and secure the top.
CAUTION3: Acetone is extremely flammable. Avoid static electricity and use a fume hood when handling.
 Allow bags to soak for 10 minutes. Repeat with fresh acetone. Pour out acetone and place bags on a wire screen to air-dry.
Exception – Roasted soybean: Due to the processing of roasted soy a modification to the extraction is required. Place roasted soy samples into a container with a top. Pour enough acetone into the container to cover the bags and secure the top. Shake the container 10 times and pour off the acetone. Add fresh acetone and allow samples to soak for twelve hours. After the soak time, pour out the acetone and place the bags on a wire screen to air-dry.
8. Spread the sample uniformly inside the filter bags by shaking and flicking the bags to eliminate clumping.
9. Place up to 3 bags on each of eight Bag Suspender Trays (maximum of 24 bags). Stack the trays on the center post of the Bag Suspender with each level rotated 120 degrees in relation to the tray below it. Place the empty 9th tray on top. NOTE: All nine trays must be used regardless of the number of bags being processed.
10. Verify that the hot water supply is on, and the drain hose is securely positioned in the drain.
11. Read the Temperature Controller on the right side of the instrument. If the temperature is higher than 20°C , cool the Vessel as follows:
12. Fill the Vessel with cold water.
13. When the Temperature Controller reads 20°C , run the Flush Procedure to drain the water.
14. Repeat steps a and b if necessary.
 (Procedure continued on next page.)

Calculations

$$\% \text{ ADF (as-received basis)} = \frac{100 \times (W_3 - (W_1 \times C_1))}{W_2}$$

Where:

W_1	=	Bag tare weight
W_2	=	Sample weight
W_3	=	Dried weight of bag with fiber after extraction process
C_1	=	Blank bag correction (running average of final oven-dried weight divided by original blank bag weight)

ADF Procedure (continued)

- If you are using Cubetainers for your chemicals, attach the AD solution hose to the Cubetainer and then to Port B on the instrument.

NOTE: If you do not want to use the Cubitainers to automatically add your solutions, you can manually fill the vessel for each procedure. First make sure that the hoses to the Cubitainers are disconnected. Follow steps 12 and 13a below. Then press START on the Keypad and immediately pour 2L of solution directly into the vessel. Please note that the solution must cover the level sensor in order for the instrument to start its operation. Once agitation begins, close the Vessel Lid and follow the rest of the procedure from step 14 to the end.

- Open the Vessel Lid and insert the Bag Suspender with bags into the Vessel and place the Bag Suspender Weight on top of the empty 9th tray to keep the Bag Suspender submerged.
- Follow the instructions on the ANKOM²⁰⁰⁰ display:
- Select ADF.
- Close the Vessel Lid.
- Confirm hot water is on (>70°C).
- Press START.
- When the ADF extraction and rinsing procedures are complete, open the Vessel Lid and remove the filter bags. Gently press out excess water from the bags. Place bags in a 250ml beaker and add enough acetone to cover bags and soak for 3-5 minutes.
- Remove the filter bags from the acetone and place them on a wire screen to air-dry. Completely dry in an oven at 102 ± 2°C. (In most ovens the filter bags will be completely dry within 2-4 hours.) Do not place bags in the oven until the acetone has completely evaporated.

NOTE: When running a lignin procedure or a sequential (NDF/ADF or NDF/ADF/Lignin) with the F57 Filter Bag it is important not to dry the bags overnight after the NDF or ADF procedure. A drying timeframe of 2-4 hours at 100°C to 105°C is sufficient to thoroughly dry the bags after each procedure. Extended drying times or too high a temperature can compromise the bag's filtration media. In addition, be sure to check the water of the fourth rinse during the ADF procedure to ensure all the sulfuric acid has been removed from the bags. If litmus paper shows the presence of acid during the fourth hot water rinse, repeat until neutral.

- Remove the filter bags from the oven and immediately place them directly into a collapsible desiccant pouch and flatten to remove any air. Cool to ambient temperature and weigh the filter bags (W_3). NOTE: Do not use a conventional desiccator container.

Neutral Detergent Fiber in Feeds - Filter Bag Technique (for A2000 and A2000I)

Definition

This method determines Neutral Detergent Fiber, which is the residue remaining after digesting in a detergent solution. The fiber residues are predominantly hemicellulose, cellulose, and lignin.

Scope

This method is applicable to grains, feeds, forages, and all fiber-bearing material.

Apparatus

1. Analytical Balance—capable of weighing 0.1 mg.
2. Oven—capable of maintaining a temperature of $102 \pm 2^\circ\text{C}$.
3. Digestion instrument—capable of performing the digestion at $100 \pm 0.5^\circ\text{C}$ and maintaining a pressure of 10-25psi. The instrument must be capable of creating a similar flow around each sample to ensure uniformity of extraction (ANKOM²⁰⁰⁰ with 65rpm agitation, ANKOM Technology).
4. Filter Bags—constructed from chemically inert and heat resistant filter media, capable of being heat sealed closed and able to retain 25-micron particles while permitting solution penetration (F57 and F58, ANKOM Technology).
5. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure (HS or HSi, ANKOM Technology).
6. Desiccant Pouch—collapsible sealable pouch with desiccant inside that enables the removal of air from around the filter bags (MoistureStop weigh pouch, ANKOM Technology).
7. Marking pen—solvent and acid resistant (F08, ANKOM Technology).

Reagents

1. Neutral Detergent Solution—Add 30g Sodium lauryl sulfate (USP), 18.61g Ethylenediaminetetraacetic disodium salt (dehydrate), 6.81g Sodium borate, 4.56g Sodium phosphate dibasic (anhydrous), and 10.0ml Triethylene glycol to 1L distilled H₂O (premixed chemical solution available from ANKOM Technology). Check that pH is from 6.9 to 7.1. Agitate and heat to aid solution.
CAUTION1: Powdered chemicals will irritate mucous membranes. A dust mask and gloves should be worn when handling these chemicals.
2. Alpha-amylase—Heat-stable bacterial alpha-amylase: activity = 17,400 Liquefon Units / ml (FAA, ANKOM Technology).
3. Sodium sulfite—Na₂SO₃, anhydrous (FSS, ANKOM Technology)

Sample Preparation

Grind samples in a centrifugal mill with a 2mm screen or cutter type (Wiley) mill with a 1mm screen. Samples ground finer may have particle loss from the filter bags and result in low values.

NDF Procedure (see the NDF Analysis section of the Operator's Manual for more detail)

1. Use a solvent resistant marker to label the filter bags to be used in the analysis.
2. Weigh and record the weight of each empty filter bag (W_1) and zero the balance. NOTE: Do not pre-dry filter bags. Any moisture will be accounted for by the blank bag correction.
3. Place 0.45 – 0.50g of prepared sample in up to 23 of the bags and record the weight (W_2) of each. Avoid placing the sample in the upper 4mm of the bag.

4. Include at least one empty bag in the run to determine the blank bag correction (C_1).

NOTE: A running average blank bag correction factor (C_1) should be used in the calculation of fiber. The inclusion of at least one blank bag in each run is mainly used as an indicator of particle loss. A C_1 larger than 1.0000 indicates that sample particles were lost from filter bags and deposited on the blank bag during the extraction. Any fiber particle loss from the filter bags will generate erroneous results. If particle loss is observed, then the grinding method needs to be evaluated.

5. Using a heat sealer, completely seal each filter bag closed within 4mm of the top to encapsulate the sample. NOTE: Use sufficient heat to completely seal the filter bags and allow enough cool time (2 sec) before removing each bag from the heat sealer.
6. Spread the sample uniformly inside the filter bags by shaking and flicking the bags to eliminate clumping.
7. **Pre-extract only samples containing >5% fat:** Extract samples by placing bags with samples into a container with a top. Pour enough acetone into the container to cover the bags and secure the top.

CAUTION2: Acetone is extremely flammable. Avoid static electricity and use a fume hood when handling.

Allow bags to soak for 10 minutes. Repeat with fresh acetone. Pour out acetone and place bags on a wire screen to air-dry.

Exception – Roasted soybean: Due to the processing of roasted soy a modification to the extraction is required. Place roasted soy samples into a container with a top. Pour enough acetone into the container to cover the bags and secure the top. Shake the container 10 times and pour off the acetone. Add fresh acetone and allow samples to soak for twelve hours. After the soak time, pour out the acetone and place the bags on a wire screen to dry.

8. Spread the sample uniformly inside the filter bags by shaking and flicking the bags to eliminate clumping.
9. Place up to 3 bags on each of eight Bag Suspender Trays (maximum of 24 bags). Stack the trays on the center post of the Bag Suspender with each level rotated 120 degrees in relation to the tray below it. Place the empty 9th tray on top. NOTE: All nine trays must be used regardless of the number of bags being processed.
10. Verify that the hot water supply is on, and the drain hose is securely positioned in the drain.
11. If you are using Cubetainers for your chemicals, attach the ND solution hose to the Cubetainer and then to Port A on the instrument.

(Procedure continued on next page.)

Calculations

$$\% \text{ NDF (as-received basis)} = \frac{100 \times (W_3 - (W_1 \times C_1))}{W_2}$$

Where:

W_1	=	Bag tare weight
W_2	=	Sample weight
W_3	=	Dried weight of bag with fiber after extraction process
C_1	=	Blank bag correction (running average of final oven-dried weight divided by original blank bag weight)

NDF Procedure (continued)

- Open the Vessel Lid and insert the Bag Suspender with bags into the Vessel and place the Bag Suspender weight on top of the empty 9th tray to keep the Bag Suspender submerged.

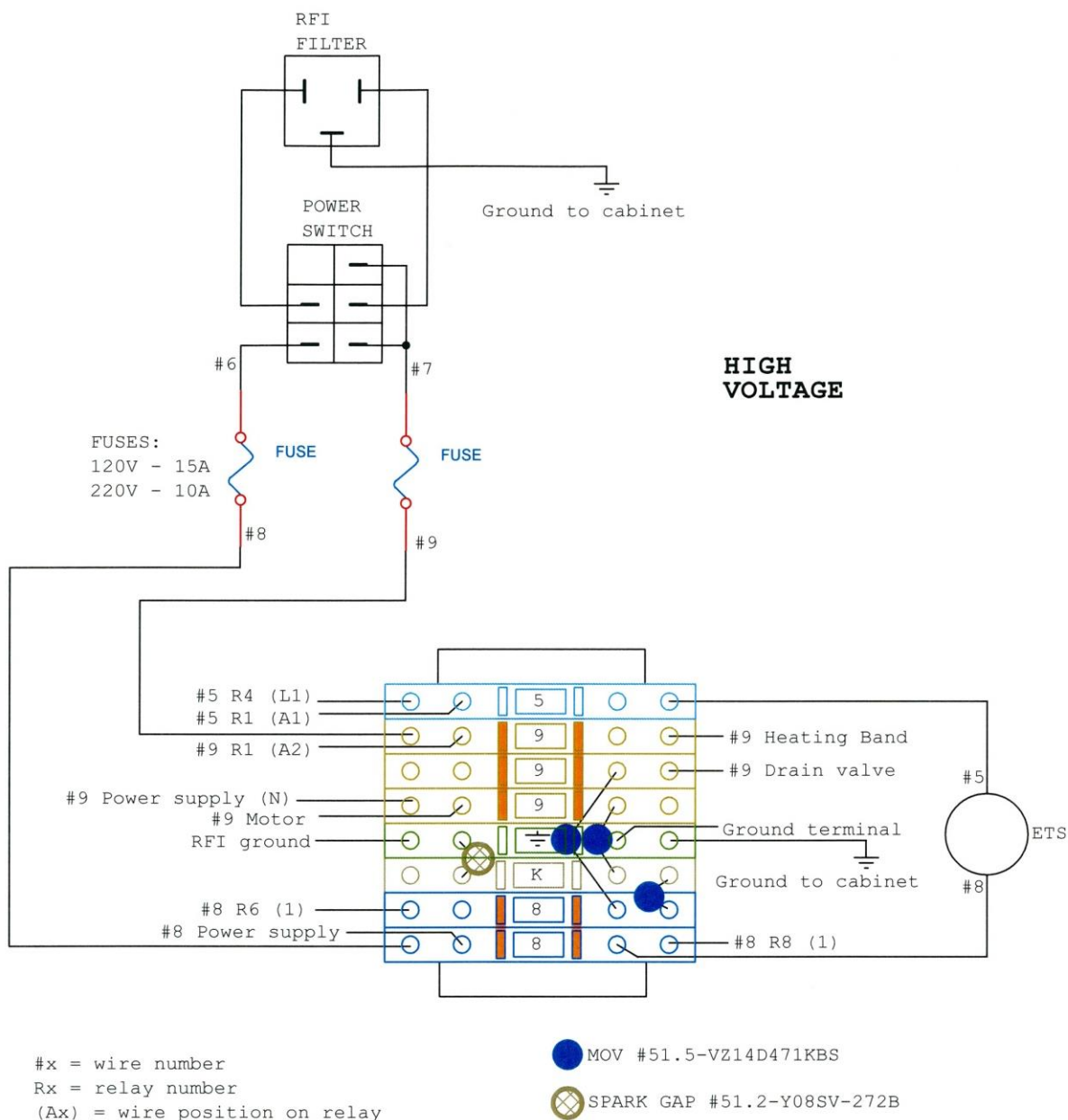
NOTE: If you do not want to use the Cubitainers to automatically add your solutions, you can manually fill the vessel for each procedure. First make sure that the hoses to the Cubitainers are disconnected. Follow steps 12a and 12b below. Then press START on the Keypad and immediately pour 2L of solution directly into the vessel. Then add 20g of Na₂SO₃ and 4.0ml of alpha-amylase. Please note that the solution must cover the level sensor for the instrument to start its operation. Once agitation begins, close the Vessel Lid and follow the rest of the procedure from step 13 to the end.

- Follow the instructions on the ANKOM²⁰⁰⁰ display:
- Select NDF. (**Wait** to close the Vessel Lid.)
- Confirm hot water is on (>70°C).
- Press START.
- After the ND solution has been automatically inserted and agitation begins, manually add 20g of Na₂SO₃ and 4.0ml of alpha-amylase.
- Close the Vessel Lid.
- Attach the Amylase Dispenser Assembly to Port B on the instrument. Add 8.0ml of alpha-amylase and enough water to fill the dispenser. The ANKOM²⁰⁰⁰ will automatically add the amylase solution to the first and second rinse.
- When the NDF extraction and rinsing procedures are complete, open the Vessel Lid and remove the filter bags. Gently press out excess water from the bags. Place bags in a 250ml beaker and add enough acetone to cover bags and soak for 3-5 minutes.
- Remove the filter bags from the acetone and place them on a wire screen to air-dry. Completely dry in an oven at 102 ± 2°C. (In most ovens the filter bags will be completely dry within 2-4 hours.) Do not place bags in the oven until the acetone has completely evaporated.

NOTE: When running a lignin procedure or a sequential (NDF/ADF or NDF/ADF/Lignin) with the F57 Filter Bag it is important not to dry the bags overnight after the NDF or ADF procedure. A drying timeframe of 2-4 hours at 100°C to 105°C is sufficient to thoroughly dry the bags after each procedure. Extended drying times or too high a temperature can compromise the bag's filtration media. In addition, be sure to check the water of the fourth rinse during the ADF procedure to ensure all the sulfuric acid has been removed from the bags. If litmus paper shows the presence of acid during the fourth hot water rinse, repeat until neutral.

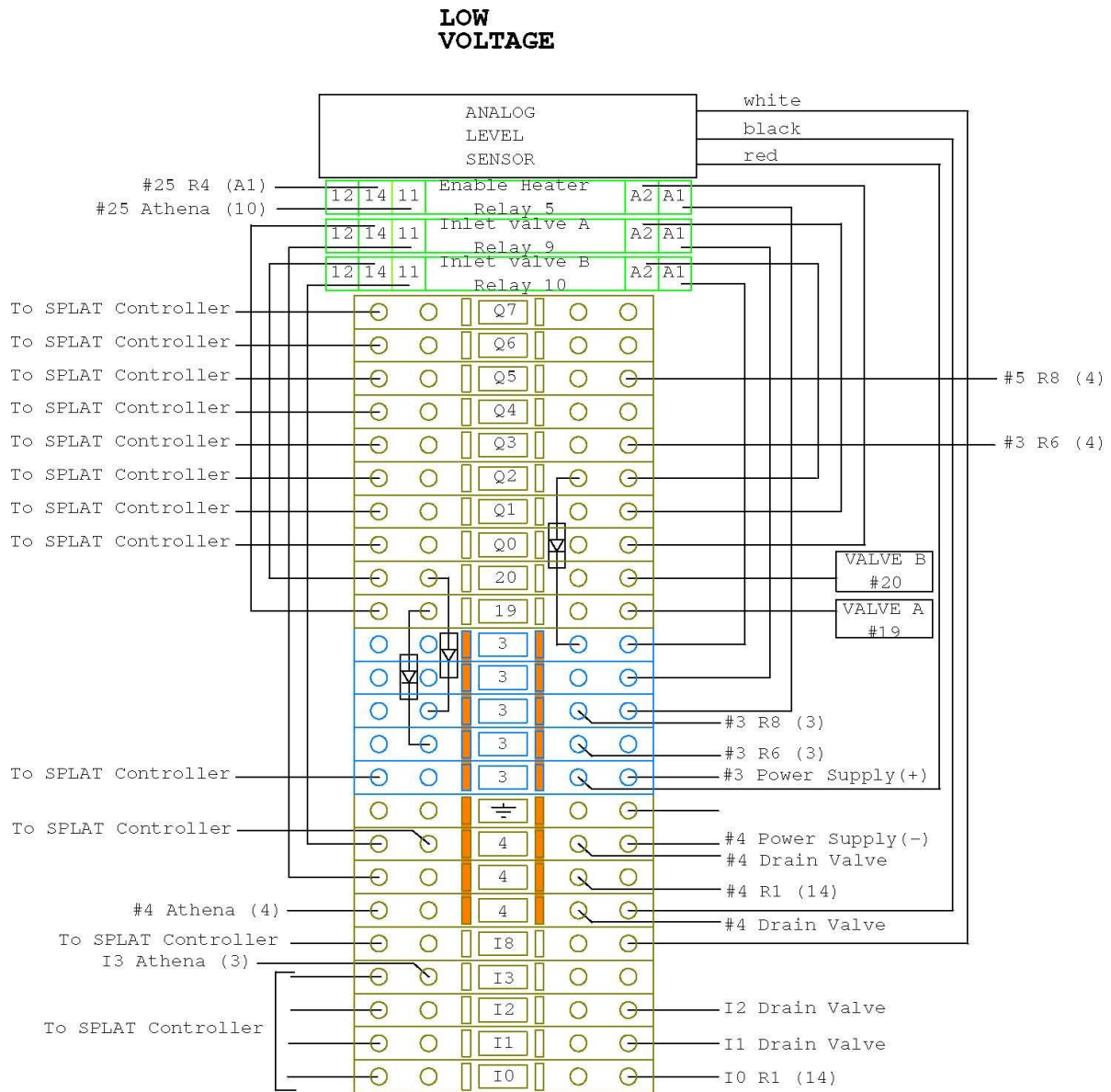
- Remove the filter bags from the oven and immediately place them directly into a collapsible desiccant pouch and flatten to remove any air. Cool to ambient temperature and weigh the filter bags (W_3). NOTE: Do not use a conventional countertop or cabinet desiccator.

Appendix B – Wiring Diagrams (1 of 4)



ANKOM Technology			
Title			
A2000 SCHEMATIC 120/220V			
Size	Document Number		Rev
			E
Date:	Tuesday, September 21, 2010	Sheet	1 of 4

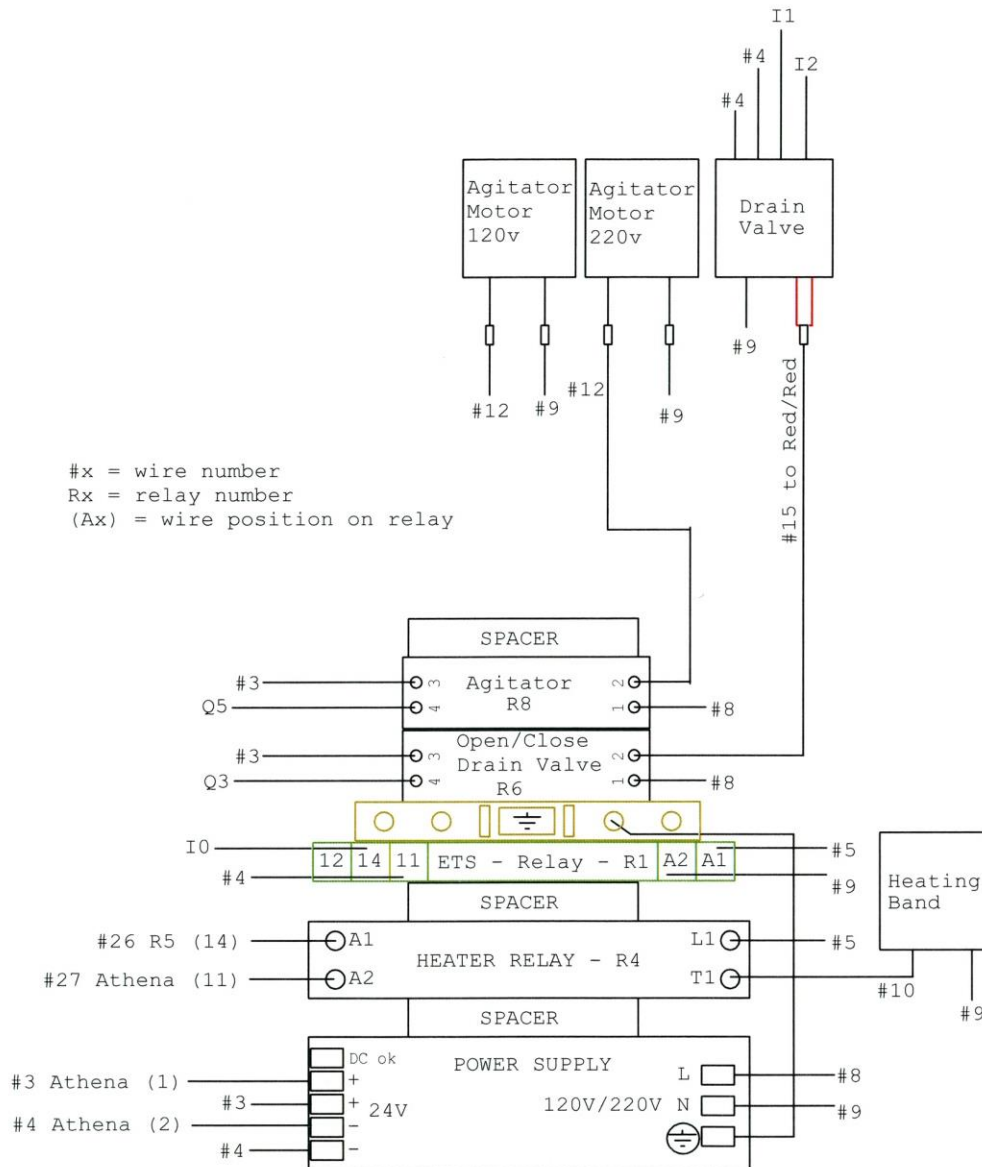
Appendix A – Wiring Diagrams (2 of 4)



#x = wire number
Rx = relay number
(Ax) = wire position on relay

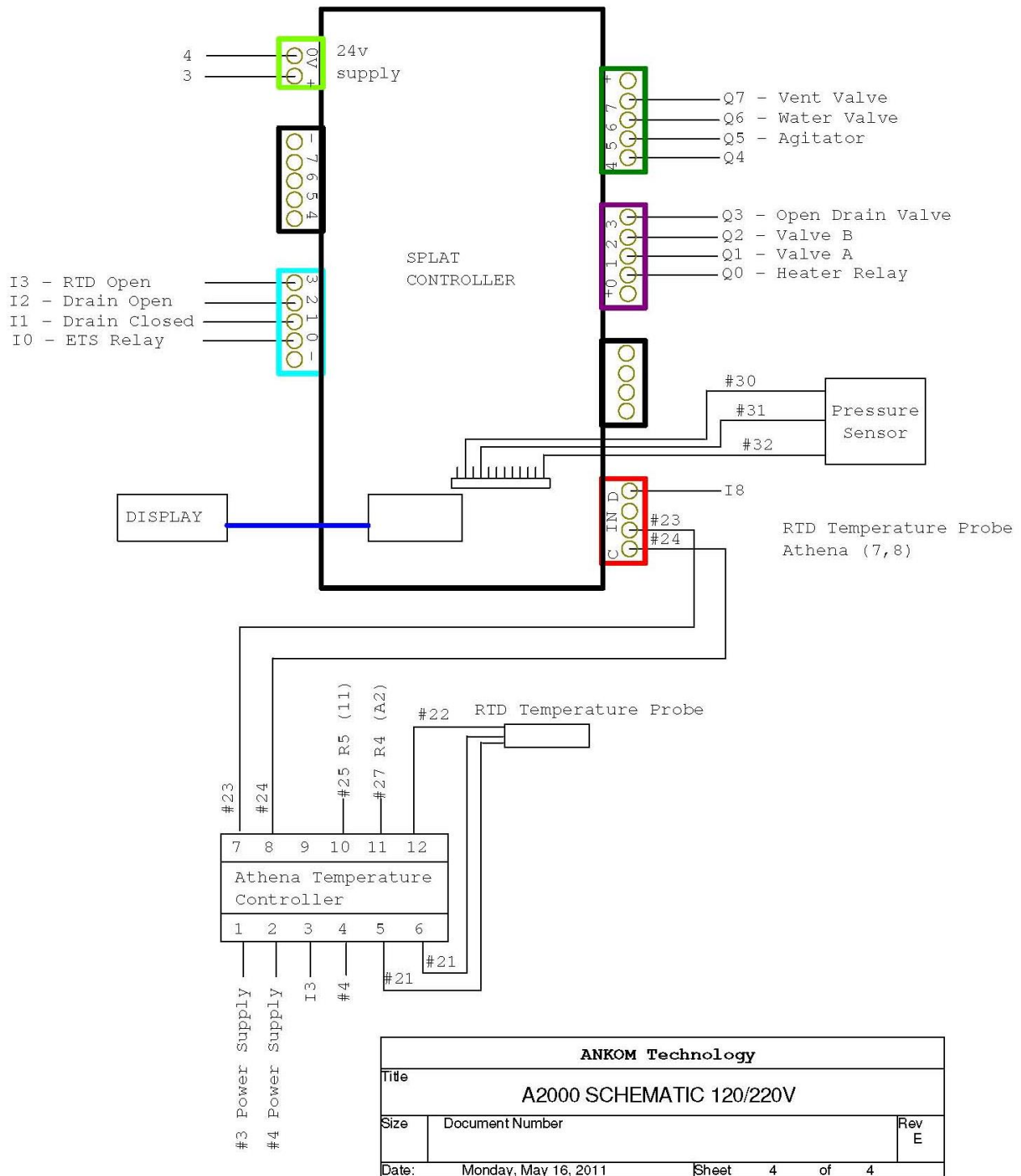
ANKOM Technology		
Title A2000 SCHEMATIC 120/220V		
Size	Document Number	Rev E
Date:	Wednesday, May 11, 2011	Sheet 2 of 4

Appendix A – Wiring Diagrams (3 of 4)







ANKOM Technology			
Title			
A2000 SCHEMATIC 120/220V			
Size A	Document Number {Doc}		Rev E
Date:	Friday, September 17, 2010	Sheet	3 of 4

Appendix A – Wiring Diagrams (4 of 4)



Automation saves time and money!

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

	<p>FLEX Analyte Extractor</p> <ul style="list-style-type: none"> • Simplifies fat-soluble vitamin and cholesterol analysis • Crude and total fat analysis capability coming soon • Eliminates chemical handling to improve safety • Eliminates bi-phase extractions • Provides ability to create custom methods
	<p>TDF Dietary Fiber Analyzer</p> <ul style="list-style-type: none"> • Automates AOAC 991.43, 985.29, 2009.01, and 2011.25 (and associated AACC methods) • IDF/SDF and TDF values • Faster, Technician-free Filtering • Computer controlled operation • Reduced per assay costs
	<p>DELTA Automated Fiber Analyzer with Pump System</p> <ul style="list-style-type: none"> • Crude Fiber (AOCS Ba 6a-05), ADF, NDF determinations • Automatically adds solutions and rinses • Batch process - up to 24 samples at one time
	<p>XT15 Fat Extractor</p> <ul style="list-style-type: none"> • Official Method AOCS Am 5-04 • Fully automatic • Solvent recovery at 97% or greater • Batch process - up to 15 samples at one time
	<p>RF Gas Production System</p> <ul style="list-style-type: none"> • High sensitivity pressure measurement • Anaerobic activity analyses (rumen, yeast, beer/wine fermentation, etc.) • Biodegradability analyses • Aerobic activity analyses (BOD, soil respiration, etc.) • Wireless Computer control and data storage

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