

Operator's Manual

ANKOM Fiber Analyzer

(A200, A200I)



ANKOM
TECHNOLOGY

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Congratulations on your purchase of an **ANKOM Fiber Analyzer**. Your selection of an ANKOM system indicates that you desire to increase your wet chemistry efficiency and maintain the highest levels of precision and accuracy possible.

The ANKOM Fiber Analyzer was designed to efficiently and accurately analyze up to 24 samples individually enclosed in filter bags. Analyses for Acid Detergent Fiber, Neutral Detergent Fiber and Crude Fiber are accomplished in a batch operation. The system can also be used to support ADIN and ADL assays and more because the filter bags are nitrogen and ash free. "Digestion" and rinse operations are all performed within the same instrument and the separate filtration step is eliminated. The ANKOM Fiber Analyzer maintains a solution temperature of 100° C, while providing proper agitation to ensure a uniform flow of chemical solution across each sample.

By ensuring the proper care and maintenance of your fiber analyzer, you will help to keep your system running smoothly for years. **Please review the entire contents of this manual before you begin operating this product.** It is also important that you follow the procedure as closely as possible. Often discrepancies in values are the result of procedural errors.

ANKOM Technology is committed to your total satisfaction. We are always available to assure that you get the most out of your ANKOM products. Feel free to contact us with your questions and suggestions. We also provide a full range of products to support *In Vitro* and *In Situ* studies. We offer chemicals and other ancillary products that will assist you in getting results at a reasonable cost.

Warranty

ANKOM Technology Corporation warrants the **ANKOM Fiber Analyzer** against any defects due to faulty workmanship or material for a one-year period after the original date of consumer purchase. This warranty does not include damage to the instrument resulting from neglect or misuse. If the instrument is damaged as a result of defects in the workmanship or materials during the warranty period, ANKOM Technology will repair or replace the instrument free of charge.

One and Three Year Extended Warranties are also available.

For any questions regarding the Warranty, Extended Warranties or Instrument, please contact ANKOM at:

Telephone (315) 986-8090

FAX: (315) 986-8091

service@ankom.com

Safety Precautions

Upper Surface:



Caution, 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.**



Caution, Hot Surfaces - Do not touch the upper surfaces of the instrument during operation as the surface temperature can exceed 70° C (160° F). **Failure to observe this caution may result in scalding or burning.**

Rear Surface:



Caution, Hazardous Voltages - Do not operate the instrument with the cover removed as there are hazardous voltages present during operation. The power cord must be disconnected prior to rear panel removal. Electrical shock or electrocution may result if ignored.



Caution Hazardous Materials - Do not operate the instrument unless the leak detector is in place. The expulsion of hazardous chemicals could result. Dispose of fluid in the same manner as the assay fluid.

General:

-All switches must be off before plugging in the instrument. **Agitate** and **Heat** buttons are in the **OFF** position when extended.

-**WARNING:** The **Heat** button should only be on when the vessel contains liquid.

-**WARNING:** Do not open the vessel lid during or after an operation until both pressure and liquid are thoroughly exhausted. After securing the exhaust hose along the path to the drain, slowly open the exhaust valve. **NOTE: Failure to secure the hose could result in uncontrolled chemical flow.**

-Three safety devices turn off the heater in the instrument in the event of a malfunction

- 1- An electric circuit breaker
- 2- A temperature shut-off switch
- 3- A pressure shut-off switch

-The user should be aware that if the equipment is used in manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Specifications

ANKOM²⁰⁰ - 110-120V~ 15 amp 50-60Hz

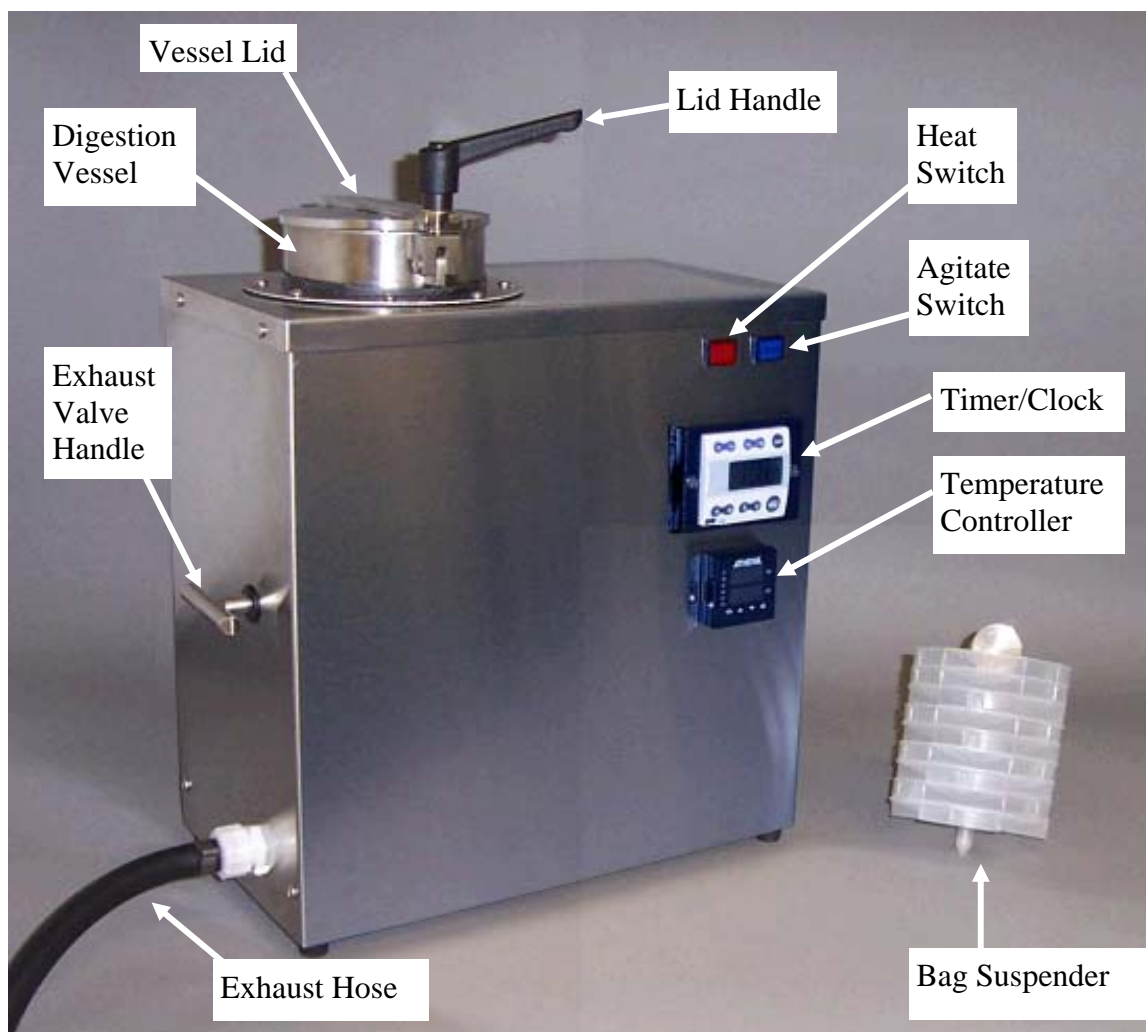
ANKOM²⁰ - 220-240V~ 8 amp 50-60Hz

Acceptable Ambient Operating Temperature - 15-30°C (60-85° F) Humidity- 20-60% RH

Instrument Set-up

Your instrument comes complete with a power cord, an exhaust hose, and a bag suspender with weight.

- Set the instrument on a firm, level surface. Place the back of the instrument no closer than three inches from a wall. **NOTE: Do not locate this instrument where it will be subject to excessive shock, vibration, dirt, moisture, oil, or other fluids. Do not place near microwave ovens or mechanical devices.**
- Plug the power cord into the instrument receptacle and then into an electrical outlet.
- The **AGITATE** button allows the agitator to aid the solution flow through the filter bags by raising and lowering the bag suspender in the vessel.
- The **HEAT** button allows the controller to heat the vessel and solution. **NOTE: Do not activate the heat unless the vessel contains an appropriate amount of solution.**
- The **Timer/Clock** has four adjustable countdown setting and a clock function. **An audible alarm will sound but will not turn off the instrument.**
- The **Controller** is preset for 100°C. Do not adjust the controller. It controls the temperature inside the digestion vessel.
- The **Exhaust Valve Handle** exhausts the pressure and solution from the vessel.
- The **Exhaust Hose** must be secured into a drain or suitable container. **NOTE: The exhausted solution is under pressure and can cause the hose end to spray in all directions.**
- The **Bag Suspender** holds up to three filter bags per tray. There are nine trays. The ninth tray acts as a cover and will not contain filter bags.



To Start the Cycle:

- Turn on the instrument's **Main Power Switch**.
- Pour a maximum of 2 liters of solution into the **Vessel**.
- Place the bag suspender with the samples and bag suspender weight into the vessel.
- Turn on the **Heat** and **Agitate** buttons. After ensuring the bag suspender is agitating, close and tighten vessel lid.
- Start the **Timer**.

To End the Cycle:

- Turn off the **Heat** and **Agitate** buttons.
- After securing the **Exhaust Hose**, slowly open the **Exhaust Valve Handle** and exhaust the solution.
- Close the **Exhaust Valve Handle**.
- Open the **Vessel Lid**.
- Pour in the rinse water as per procedure instructions, lower the lid and activate the agitation.
- Agitate for three to five minutes, rinse and exhaust the water, and repeat as per procedure.
- Remove the **Bag Suspender** from the vessel. Turn off the **Main Power Switch**.

NOTE: See Appendix D for the Boil test procedure. Perform the procedure for a baseline boiling temperature before operating the instrument. Record the temperatures in the manual for reference.

Initial/Periodic Maintenance

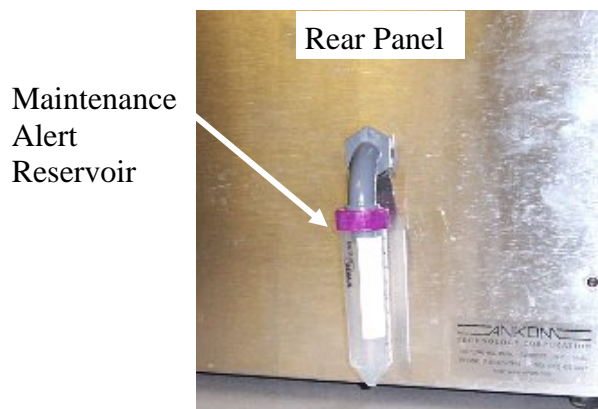
-During the first 20 hours of operation more frequent inspection of the agitator assembly is required.

- After every three to four hours of use, check the **Reservoir** of the **Maintenance Alert** for any fluid accumulation. If any fluid is present, unscrew the **Reservoir** and drain the fluid. Replace the **Reservoir**.
- Disconnect the power supply and remove the back of the instrument. Visually inspect the agitator assembly, specifically the area between the **Maintenance Alert Collector** and the **Packing Nut**. If signs of leakage are evident, clean the area thoroughly.
- Reconnect the power supply. Turn on the **Agitate** button and tighten the **Packing Nut** until the motor begins to labor (the motor sound will change). At this point, loosen the Packing Nut approximately 1/16 turn or until the motor noise returns to normal. Replace the rear panel. Check the **Maintenance Alert Reservoir** after the next assay for leakage.

NOTE: Check the packing after each month of use after the Initial Maintenance.



Interior Rear Fiber Analyzer



Initial/Periodic Maintenance (Continued)

The bag suspender and agitation should be checked periodically for proper operation. Depending on usage, check every three to six months or if fiber values are higher than normal or inconsistent. Poor agitation will cause higher values and poor repeatability.

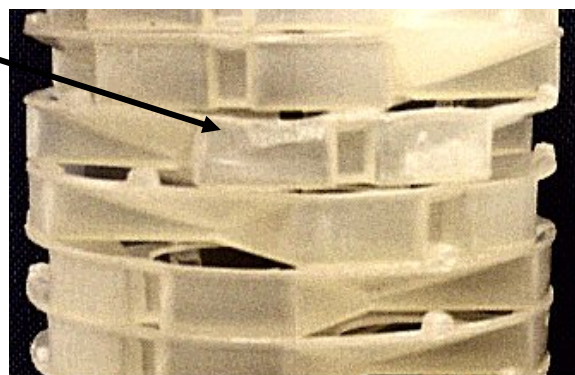
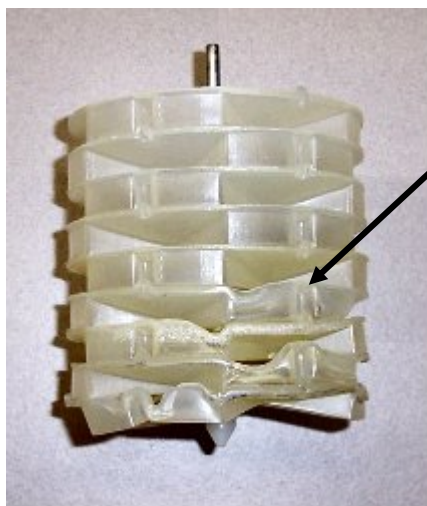


The bag suspender should have a stroke of 1/2" or 1.25 cm. If the stroke is less than 1/2" or 1.25cm, the agitator disc has flattened and a new agitator is necessary.



Place a straight edge across the top of the vessel. Turn on the **Agitate** button. The weight should not hit the straight edge at the top of the stroke. If the weight is hitting the lid or traveling above the top of the vessel, the agitator has moved up in the vessel and must be completely seated at the bottom of the vessel.

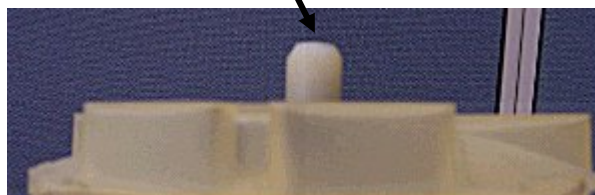
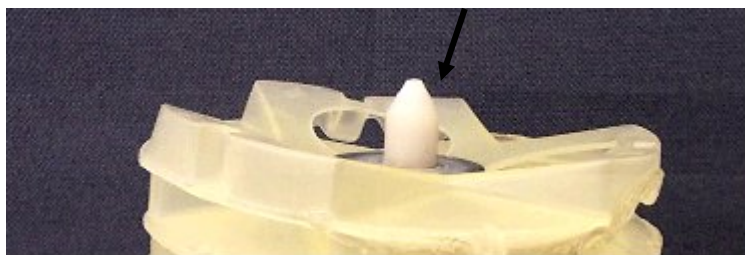
Check the trays for melting. This is an extreme example. Replace any melted or worn trays.



Check the bottom tray. It must be flat. If it is concave the bag suspender will catch in the vessel and melt. Check the tip for excessive wear and replace when worn.

Extremely concave bottom tray
Good tip

Worn tip



Storage

- When the instrument will not be in use for a long period of time (+ one month), fill the vessel with water and run the agitator for approximately 10 minutes. Rinse the vessel thoroughly out with water. This will ensure that no acid residue will corrode the instrument during storage.
- To maintain the outside cabinet, wipe the outside of the instrument with window cleaner or a retail cleaner. Acid residue left on the exterior may damage external electrical components.

Troubleshooting

Your ANKOM instrument has been designed for long service and ease of maintenance. Most problems are easily diagnosed and fixed. If you experience problems that are not addressed in the information below or if you have any questions, contact ANKOM Technology for assistance. We are committed to fast and reliable service.

Problem: The Fiber Analyzer will not turn on.

Possible Causes:

- The power cord is not plugged into the unit or the power source.
- The main power switch is not turned on.
- One of the system circuit breakers needs to be reset.

Problem: Fluid is observed in the **Maintenance Alert Reservoir**.

Possible Causes: Leakage has occurred at the agitator shaft. The **Packing Nut** inside the system requires attention. Refer to the Maintenance section of this manual for instructions.

Problem: The agitator is not moving and/or the motor sounds strained.

Possible Causes: The **Packing Nut** may have been tightened too much during periodic maintenance. Loosen the **Packing Nut** slightly until the motor begins to operate and the agitator moves freely.

If periodic maintenance was ignored and acid solution was allowed to leak into the packing assembly the shaft may become “frozen”. Check for corrosive buildup. The **Packing Set** and **Agitator** may need to be replaced.

Controller Problems

Problem: After some time, the temperature settings and the actual temperature do not match. The actual temperature is higher or lower than the factory setting.

Possible Cause: The temperature controller or temperature probe is malfunctioning.

Carefully pull the temperature controller out of the instrument by grasping both sides and pulling straight out. Wiggling from side to side may be necessary. Firmly re-insert the controller, repeat four times. This will clean the controller contacts. Check the controller function.

To check controller function: Pour about 1600 ml of water into the vessel. Leaving the vessel lid closed but not tightened, turn on the **Heat** and **Agitate switches** and allow the system to come to a boil. **NOTE: The water should begin to boil in about 12-15 minutes. Do not let the water boil over.** Once the water begins to boil, check the temperature of the water using a calibrated thermometer. Measure the water temperature near the temperature probe at the bottom of the **Digestion Vessel**. The temperature readout should be approximately 100°C. If the readings do not match, refer to the Appendix for the controller menu set up procedure and controller calibration procedure. Contact ANKOM Technology for assistance.

Problem: Temperature readings on controller fluctuate.

Possible Cause: Contamination on controller contacts. Carefully pull the temperature controller out of the instrument by grasping both sides and pulling straight out. Wiggling from side to side may be necessary. Firmly re-insert the controller, repeat four times.

Assay Problems

For problems relating to the results of your analyses using the ANKOM Fiber Analyzer first check to ensure the procedures sent with your machine were followed. Some possible variables that may affect your results are:

General problems

- ND chemicals can come out of solution. Heat and stir to create a homogeneous solution.
- Oven temperature is too hot (102-105C+/- 2C).
- Modifying the settings on the controller
- Improper weighing technique. See weighing technique in the Appendix of this manual.
- Improper or non-use of the desiccant pouch. A standard desiccator will introduce moisture in the samples as it is being opened and closed. This will cause poor precision.

Low Values

- Grind is too fine.
- Bag is not completely sealed.
- Broken seam in the bag is allowing fiber to escape.
- Digestion run too long (ADF & Crude Fiber).

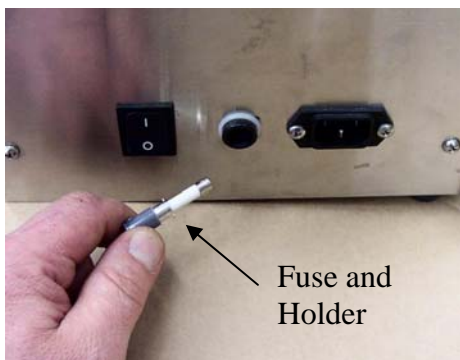
High Values

- The movement of the Bag Suspender is less than ½” up and down.
- Solution is not heated sufficiently- perform ANKOM Boil Test.
- ND – Not using Alpha Amylase and/or Sodium Sulfite.
- AD/ND-Sample too high (> 5%) in fat and is not pre-treated with solvent.
- Crude Fiber-always pre-soak with acetone.
- Oven temp low, wet samples introduced next to dry samples-samples are not sufficiently dried.
- Lack of or incorrect use of desiccant pouch- check for holes in pouch and dry packets regularly.
- Rinse water not hot enough (80° C +) or not long enough (5 minutes).
- Digestion not run long enough.
- Acetone re-used for removing water from bags post extraction.
- Improper calculation of blank bag correction or using the wrong values in the formula.
- Bag sealed too low.
- Too much sample in the bag.
- Vessel lid not closed tightly enough.

Changing the Fuse

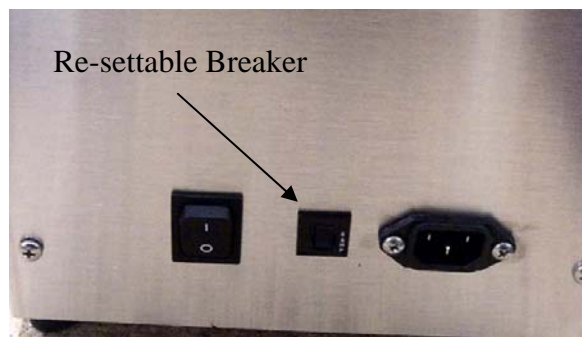
120V-

1. Turn the power off.
2. Using a flat blade screwdriver, push in and twist clockwise to open the fuse holder.
3. Replace with a 15 amp glass or ceramic fuse.
4. Reinstall the fuse and fuse holder.



220V-

1. Turn off the power.
2. Push the re-settable breaker in.
If it will not reset,
replace the breaker.



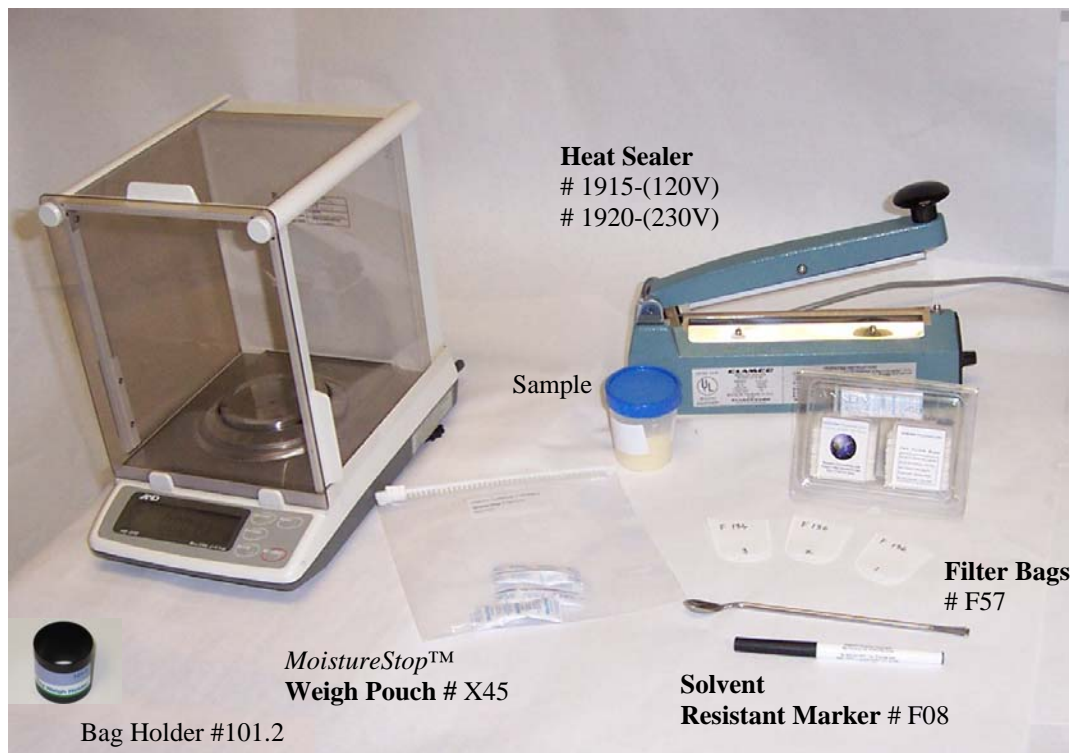
Appendix A

Weighing Procedure

ANKOM Technology W-1 Fiber Weighing Procedure: For NDF, ADF and Crude Fiber Determinations

Electronic Balance-

We recommend a four place readout on the balance and suggest “Balance Talk™” or other LIMS software for data input and management.



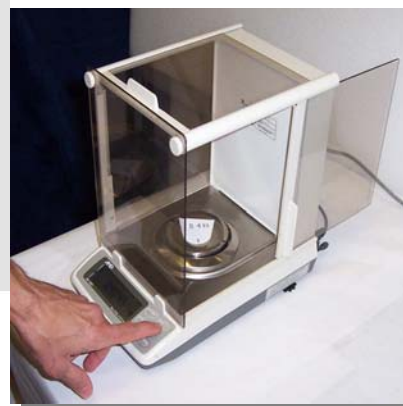
1. Number all bags using a solvent resistant marker.



2. Weigh and record filter bag weights (W1).

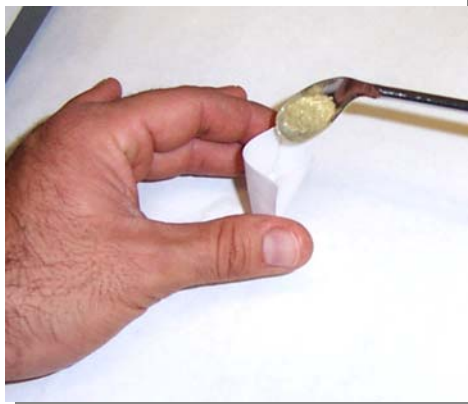


3. Tare weight of Filter Bag and holder.



Appendix A (Continued)**Weighing Procedure** (Continued)

4. Add 0.5g (for NDF &ADF) or 1.0g (for CF) of sample to bag using a spatula. Be careful not to get sample particles on the top edge of the bag sealing area.



5. Weigh sample and record its weight (W₂).



6. Heat sealer dial should be set between 4 and 5. The setting may vary from sealer to sealer.



7. Seal each Filter Bag within 4mm of its open end. Hold down handle for 2-3 seconds after the red light turns off to cool seal.



8. IMPORTANT-The seal can be seen as a solid melted stripe along the top edge. If the seal is not strong, re-seal.



9. Stack each tray on the Bag Suspender rod. Insert weighed Filter Bags into each Bag Suspender tray as shown. Perform the extraction.



11. After fiber extraction is complete, acetone rinse and dry according to the procedure. Remove from the oven and immediately store in the *MoistureStop* weigh pouch.
12. Cool to room temperature and re-weigh each bag (W₃).



$$\text{As-Is Basis- } \frac{(W_3 - (W_1 \times C_1)) \times 100}{W_2}$$

$$\text{DM Basis- } \frac{(W_3 - (W_1 \times C_1)) \times 100}{W_2 \times \text{DM}}$$

$$\text{OM DM Basis- } \frac{(W_4 - (W_1 \times C_2)) \times 100}{W_2 \times \text{DM}}$$

Where:

W₁=bag tare weight

W₂= sample weight

W₃=weight after extraction


W₄=weight of organic matter (OM) (loss of weight on ignition of bag and fiber residue)










C₁=Blank bag correction(final oven-dried weight/original bag weight)

C₂=Ash corrected blank bag (loss of weight on ignition of blank bag/original blank bag weight)

Controller Procedures








STEPS TO ACCESS MENUS AND PROGRAM CONTROLLER (Purchased Post 9/03)

If you have a controller problem, these menu settings are for reference. Contact ANKOM Technology for assistance. Your controller is preset at the factory. To set (or reset) the controller, follow the steps outlined below. The menu Ac.Cd 05 is set first, then Ac.Cd 02, then Ac.Cd 03. To make the settings permanent after the menus are completed, **set Ac.Cd to 01., then push** 

1. Push  until “Ac. Cd” flashes. Let  go.
2. Use  to  display “Ac.Cd 05”.
3. Push  once- “SnSr” will flash and alternate with the “d” setting. Use  or  to change the setting. The settings should match those listed below.
4. Push  once to reach the next setting. Each push will change the setting once. If you miss one you must start the sequence again. To do this, press  until the first setting of the menu appears again.

Ac. Cd 05 (Menu)

<u>SETTING</u>	<u>CODE</u>
SnSr	d
Out1	Ht.P
Filt.	.5
Out2	CL.O
CoL.t	H2o
A1.H.L.	HI
A1.P.d.	Pr
A1.O.P.	OFF
A2.H.L	Lo
A2.P.d.	Pr
A2.O.P.	OFF
Unit	C

5. When the Ac.Cd 05 menu is completed, push  then  Use  or  to display “Ac. Cd 02”.
- Push the  button to advance to the next menu setting.  Or  are used to change the settings.





Ac.Cd 02 (menu)

<u>SETTING</u>	<u>CODE</u>
Gn.o1	250
rAtE	25
rSEt	120
C.HYS	02
C.SP.r	15
dPnG	nL

6. Repeat actions indicated on Step 5 to get to “Ac. Cd 03” and program as follows.

Ac.Cd 03(menu)

<u>SETTING</u>	<u>CODE</u>
CY.t1	15
SP.tt	Off
L.SP.L	0
U.SP.L	105

7. Push , then  to get to the “Ac.Cd” display. Push  until “Ac.Cd 01” is displayed. Press 

The controller settings are now locked in and cannot be changed without going through the menu procedure.

8. Use the  or  to change the temperature settings to 100.0 (green display).

Locate the Fiber Analyzer away from microwave ovens or any other electromechanical devices. Electronic interference may change the controller settings.

Controller Procedures (Continued)

ANKOM 200/220 Boil Test Procedure: (Ensure the controller readings are normal)

The current temperature will show on the top display. The bottom display will read 100.0 C. Place approximately 1800 mL of water into the ANKOM digestion vessel. Do not include the bag suspender. Activate the HEAT and AGITATE buttons. Close the lid, but do not tighten. Occasionally stir the water to ensure uniform heating. **The water should begin to boil in 12 - 15 minutes.**

Record the amount of time it takes to get to the initial boil and the temperature on the controller when a strong boil is reached. (**Note:** The bottom controller readout should be set at 100.0 C.) If a boil does not occur between 99.2 and 100.5 C, use the procedure below to adjust the calibration.

Five minutes after reaching boil, stir the water and record the temperature.

Results:

- Time to boil _____
- Controller temperature at initial boil _____
- Controller temperature after five minutes of boil _____

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ANKOM Technology Controller Temperature Calibration

To recalibrate the temperature of the controller:

- Fill the instrument with 2 liters of water.
- Bring to boil using Heat and Agitate.
- Press the far left key pad for 5 seconds to access the AC menus.
- Use the arrow key pads to reach AC 04.
- Press the far left key pad and find CAL. H.
- Stir the water vigorously.
- Measure the water temperature using a calibrated thermometer. Measure next to the temperature probe in the vessel. This will be your reference temperature.
- Use the arrow key pads to set the calibration to the thermometer's reading.
- Press the far right key pad, then the far left button to find AC again. Use the arrows to get to AC 01.
- Press the far right key pad to lock the controller out.

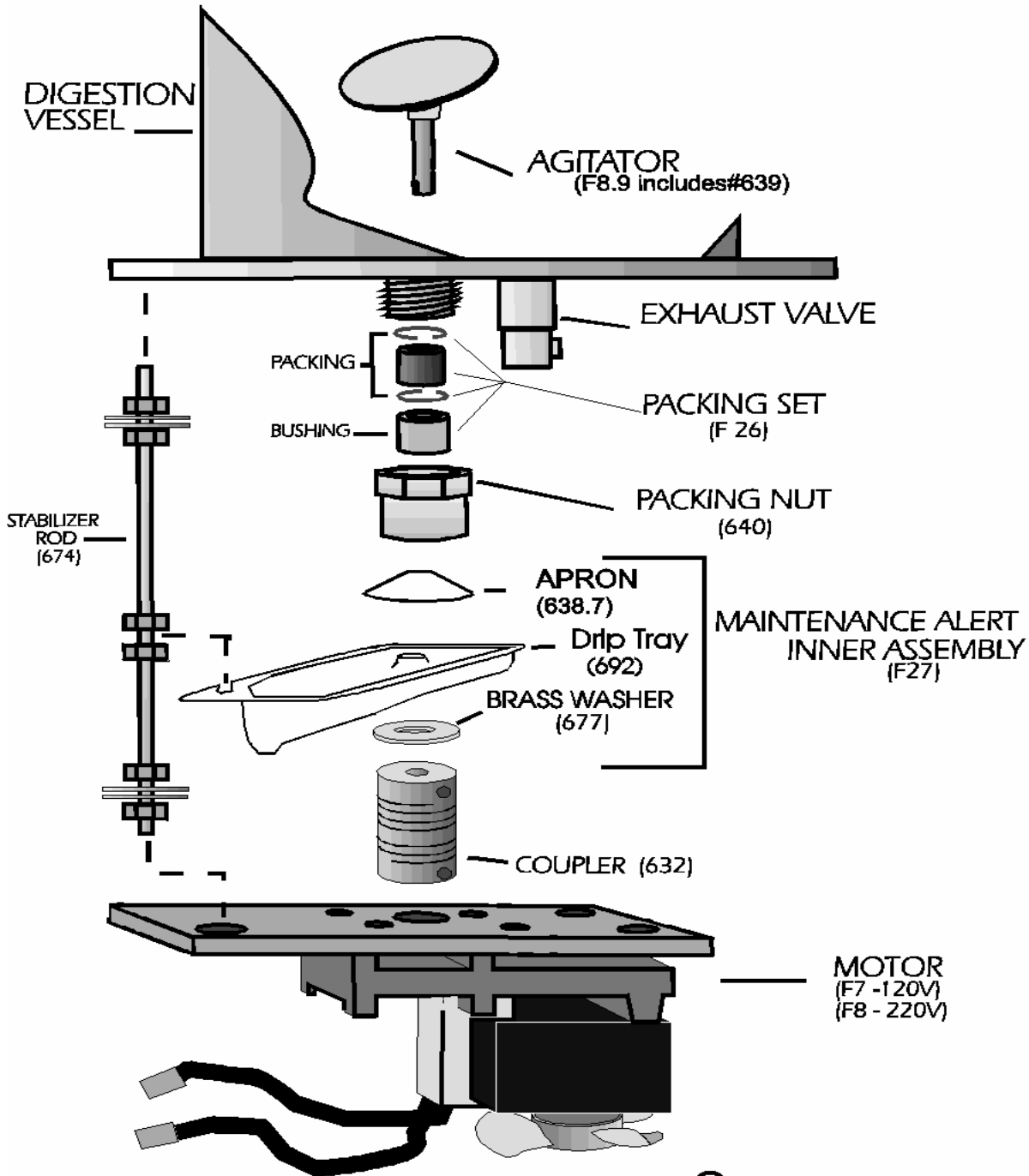
This should calibrate the controller to the probe. The water should now boil at the calibrated temperature and maintain the temperature properly.

Appendix C

Assemblies and Parts

A200, A200I

AGITATOR AND MAINTENANCE ALERT ASSEMBLY 9/23/02



Appendix C (Continued)**Assemblies and Parts** (Continued)

F1
Temperature
Probe Assembly
Instructions-F1
1 lb



F3
Hinge Block
Assembly
1 lb



F4
Lid Assembly
3 lb



F4.5
O-ring Assembly
Instructions-F4.5
1 lb

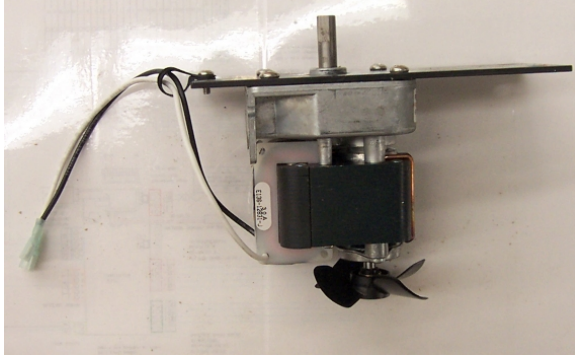


F4.8
Lid Handle
Assembly
1 lb

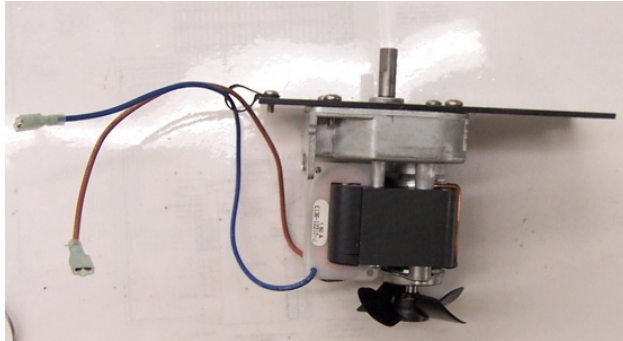


Assemblies and Parts (Continued)

F7
120 Motor Assembly
Instructions-F7/F8
4 lb



F8
220 Motor Assembly
Instructions-F7/F8
4 lb



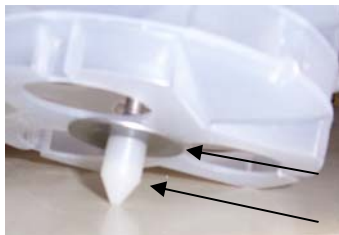
F8.9
Agitator Assembly
Instructions-F8.9
1 lb



F11
Bag Suspender Assembly
With Weight
2 lb



F11.5
Kynar Tip Assembly
1 lb



Assemblies and Parts (Continued)

F 12
Outer
Exhaust Hose Assembly
1 LB



F 12.5
Bag Suspender Rod
Assembly
1 LB

F 16
120v Fuse Assembly
Instructions-F16
1 LB



F 17
Timer Assembly
1 LB



F 18
Heat Switch
Assembly
1 LB



F 19
Agitate Switch
Assembly
1 LB



Assemblies and Parts (Continued)

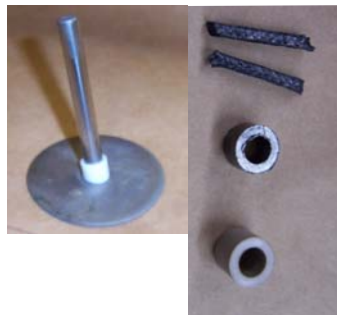
F 26
Packing Assembly
Instructions-F26
1 LB



F 27
Maintenance Alert
Inner Assembly
Instructions-F27
1 LB



F 28
Agitator Packing
Assembly
Instructions-F28
1 LB



F 29
Controller
Assembly
1 LB



Assemblies and Parts (Continued)

#603 Bolt Shoulder



#693 Bag Suspender Tray (Bottom)



#704 Lamp



#694 Bag Suspender Tray (Top)



#639.1 Bag Weight Machined



Relay
#629(120)
#630(220)

A200/A200I/A220 Left Inside View of Cabinet

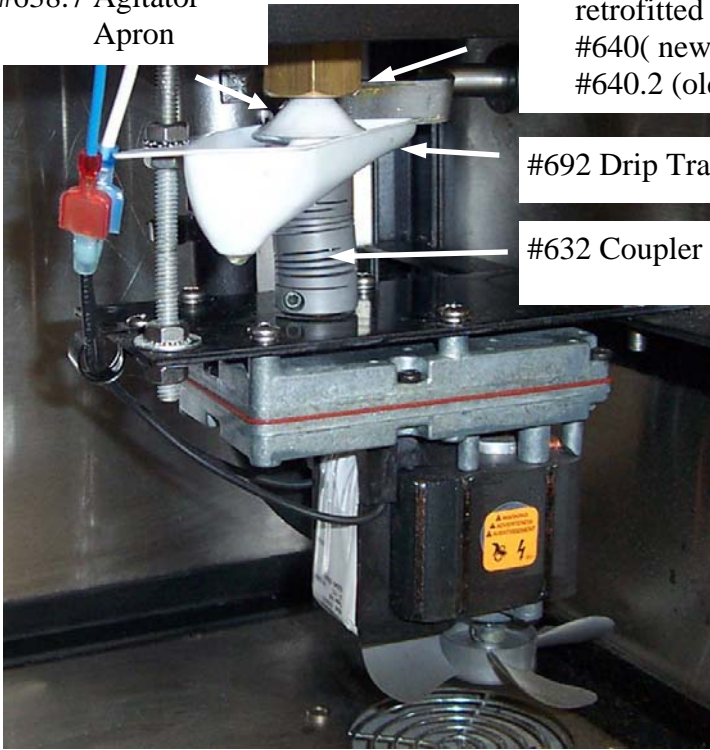


Pressure Switch F13

#622 ETS Snap Disc

A200/A200I/A220 Rear Inside View of Vessel

#638.7 Agitator Apron

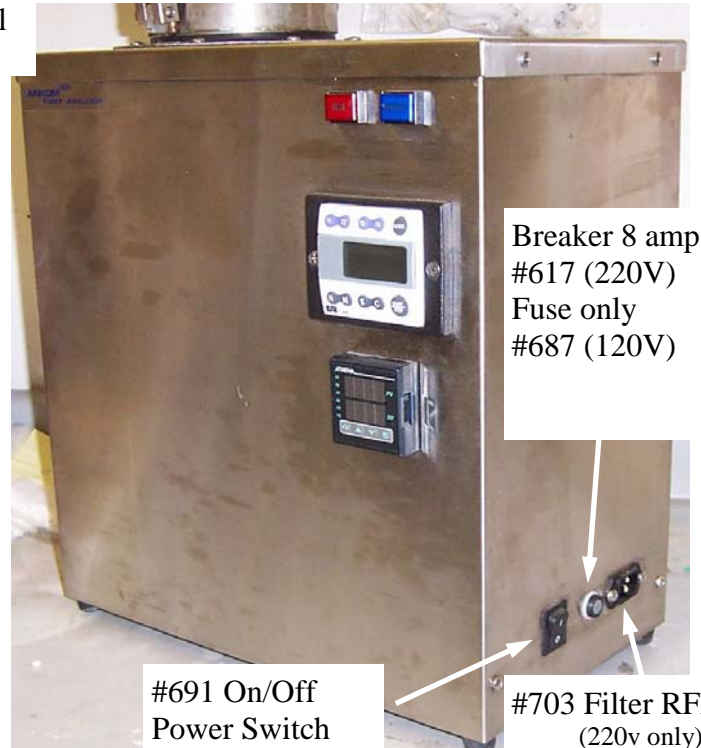


#692 Drip Tray

#632 Coupler Helical

Brass Packing Nut– Ask for serial number, and if instrument been retrofitted
#640(new) After #215, 3102
#640.2 (old) Before #215, 3102

A200/A200I/A220 Rear Inside View, right side

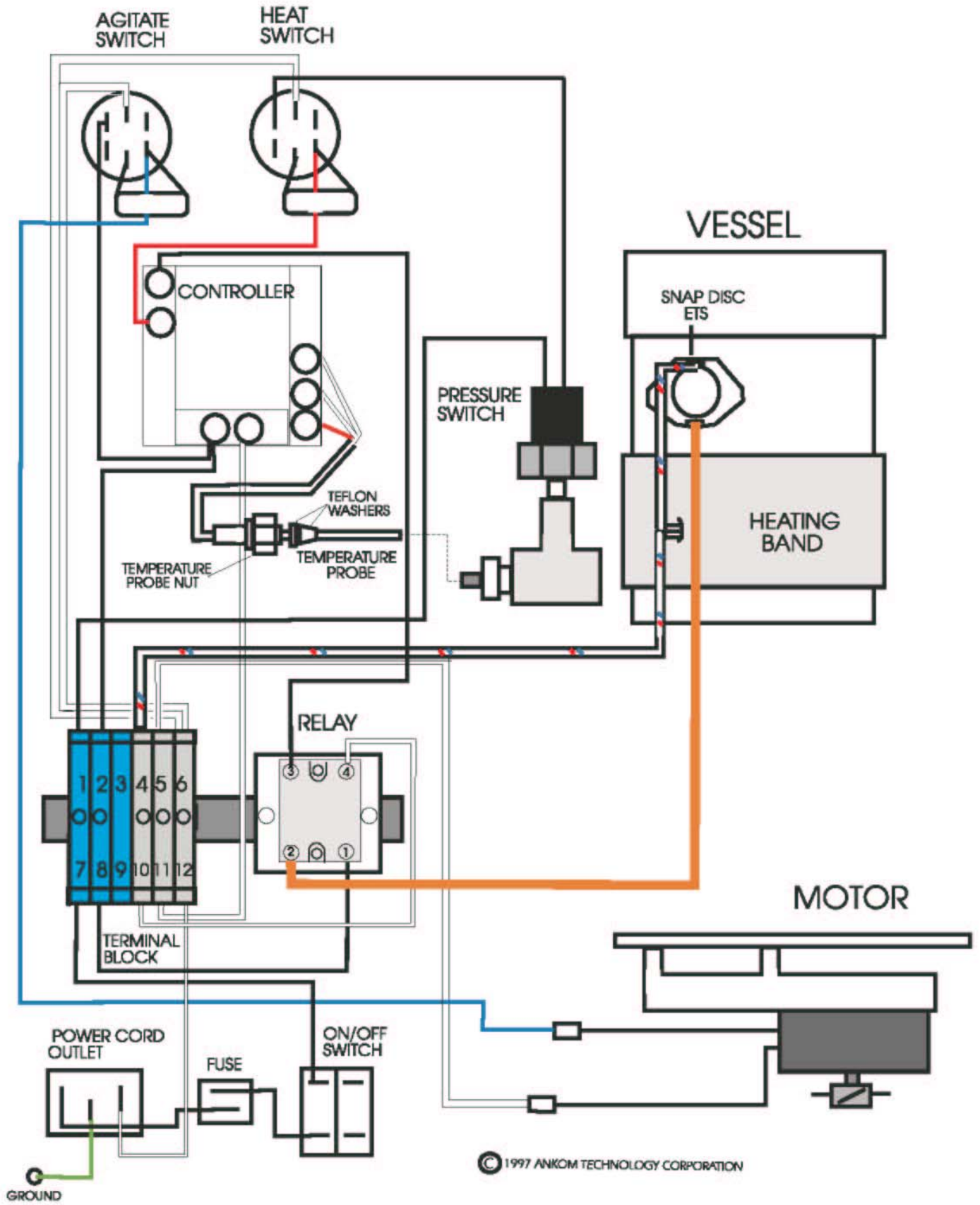


Breaker 8 amp
#617 (220V)
Fuse only
#687 (120V)

#691 On/Off Power Switch

#703 Filter RFI (220v only)

Wiring Diagram



Acid Detergent Fiber in Feeds Filter Bag Technique

(For A200, A200I)

DEFINITION

This method determines Acid Detergent Fiber, which is the residue remaining after digesting with H_2SO_4 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 $102 \pm 2^\circ C$.
3. Digestion instrument—capable of performing the digestion at $100 \pm 0.5^\circ C$ and maintaining a pressure of 10-25 psi. The instrument must also be capable of creating a similar flow around each sample to ensure uniformity of extraction (ANKOM²⁰⁰, 65 rpm 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 rapid solution penetration (F57, ANKOM Technology).
5. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure (1915, ANKOM Technology).
6. Desiccator 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 1 L 1.00N H_2SO_4 previously standardized (premixed chemical solution available from ANKOM). Agitate and heat to aid solution. (see Notes, *Caution*).

PREPARATION OF SAMPLE

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

PROCEDURE

1. Use a solvent resistant marker to label the filter bags. Weigh filter bag (W_1) and zero balance.

Note—Do not pre-dry filter bags; any moisture will be accounted for by the blank bag correction.

2. Weigh 0.45-0.55 g of prepared sample (W_2) directly in filter bag. Avoid placing the sample on the upper 4 mm of the bag.
3. Using a heat sealer, completely seal the upper edge of the filter bag within 4 mm of the top.

Note—Use sufficient heat to completely seal the filter bag and allow enough cool time (2 sec) before removing the bag from the heat sealer.

4. Weigh one blank bag and include in run to determine blank bag correction factor (C_1 , see Number Note 1).
5. **Pre-extract only samples containing soybean products or >5% fat:** Extract samples by placing 24 bags with samples into a container with a top. Pour enough acetone into container to cover bags and secure top. Shake the container 10 times and 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 container to cover bags and secure top. Shake the container 10 times and pour off acetone. Add fresh acetone and allow samples to soak for twelve hours. After soak time, pour out acetone and place bags on a wire screen to air-dry.

6. Place a maximum of 24 bags into the Bag Suspender. All nine trays should be used regardless of the number of bags being processed. Place three bags per tray and then stack trays on center post with each level rotated 120 degrees. Insert the Bag Suspender with bags into the fiber analyzer vessel and place the Bag Suspender weight on top to keep it submerged.

Note—Prior to inserting the Bag Suspender, if the vessel temperature is warm from a previous run, add cold water and exhaust.

7. When processing 24 sample bags, add 1900-2000 mL of ambient temperature AD solution to the fiber analyzer vessel. If processing less than 20 bags, add 100 mL/bag of AD solution (use minimum of 1500 mL to ensure Bag Suspender is covered).
8. Turn Agitate and Heat ON and confirm agitation. Set timer for 60 min and close lid.
9. At end of extraction, turn Heat and Agitate off. Open the drain valve (slowly at first) and exhaust hot solution before opening lid.
Note—The solution in the vessel is under pressure. The exhaust valve needs to be opened to release the pressure and solution prior to opening the lid.
10. After the solution has been exhausted, close the exhaust valve and open the lid. Add 1900-2000mL of (70-90°C) rinse water. Turn Agitate on and rinse for 5 min. The lid may be sealed with the Heat on or left open with the Heat off. Repeat 5 min. hot water rinses a total of three times or until water is neutral pH.
11. When the rinsing process is complete remove the samples. Gently press out excess water from bags. Place bags in a 250 mL beaker and add enough acetone to cover bags and soak for 3-5 min.
12. Remove bags from acetone and place on a wire screen to air-dry. Completely dry in oven at 102±2°C (most ovens will complete drying within 2-4 hrs).

Note—Do not place bags in the oven until acetone has completely evaporated.

13. Remove bags from oven, place directly into a collapsible desiccant pouch and flatten to remove air. Cool to ambient temperature and weigh bags.

Note—Do not use conventional desiccator container.

CALCULATIONS

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

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 factor (running average of final oven-dried weight divided by original weight)

NOTES

Caution

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.

CTAB will irritate the mucous membranes. A dust mask and gloves should be worn when handling this chemical.

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

NUMBERED NOTES

1. A running average blank bag correction factor (C_1) should be used in the calculation of fiber. The inclusion of a 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. Any fiber particle loss from the filter bags will generate erroneous results. If particle loss is observed then grinding method needs to be evaluated.

Crude Fiber Analysis in Feeds By Filter Bag Technique (For A200, A200I)

DEFINITION

This method determines crude fiber which is the organic residue remaining after digesting with 0.255N H₂SO₄ and 0.313N NaOH. The compounds removed are predominantly protein, sugar, starch, lipids and portions of both the structural carbohydrates and lignin.

SCOPE

This method is applicable for all feed materials such as grains, meals, pet foods, mixed feeds, forages and the following oilseeds: corn and soybeans.

APPARATUS

1. Analytical Balance—capable of weighing down to 0.1 mg.
2. Oven—capable of maintaining a temperature of 102±2°C.
3. Electric muffle furnace—with rheostat control and pyrometer that will maintain a temperature of 600±15°C
4. Digestion instrument—capable of performing the digestion at 100±0.5°C and maintaining a pressure of 10-25 psi. The instrument must also be capable of creating a similar flow around each sample to ensure uniformity of extraction. (ANKOM²⁰⁰ w/65 rpm agitation, ANKOM Technology).
5. 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 rapid solution penetration (F57 or F58, ANKOM Technology, see Numbered Notes 1).
6. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure (1915, ANKOM Technology).
7. Desiccator pouch—collapsible sealable pouch with desiccant inside that enables the removal of air from around the filter bags (MoistureStop Weigh Pouch, ANKOM Technology).
8. Marking pen—solvent and acid resistant (F08, ANKOM Technology).

REAGENTS

1. Sulfuric acid solution—0.255±0.005N. 1.25 g H₂SO₄/100 mL. Concentration must be checked by titration (see Notes, *Caution*).
2. Sodium hydroxide solution—0.313±0.005N. 1.25 g NaOH/100 mL. Concentration must be checked by titration (see Notes, *Caution*).

PREPARATION OF SAMPLE

Grind samples through a centrifugal mill with a 2 mm screen or cutter type (Wiley) mill with a 1 mm screen.

Samples ground finer may show particle loss from the filter bags and result in low values.

PROCEDURE

1. Use a solvent and acid resistant marker to label the filter bags. Weigh filter bag (W₁) and zero balance.

Note—Do not pre-dry filter bags; any moisture will be accounted for by the blank bag correction.

2. Weigh 0.95-1.00 g of prepared sample (W₂) directly in filter bag. Avoid placing the sample on the upper 4 mm of the bag.
3. Using a heat sealer, completely seal the upper edge of the filter bag within 4 mm of the top.

Note—Use sufficient heat to completely seal the filter bag and allow enough cool time (2 sec) before removing the bag from the heat sealer.

4. Weigh one blank bag and include in run to determine blank bag correction (C₁, see Number Note 2).
5. Extract fat from samples by placing all bags into a 250 mL container. Add enough petroleum ether to cover bags and soak for 10 min. Pour off solvent and allow bags to air-dry. Spread sample uniformly inside the filter bag by shaking and flicking the bag to eliminate clumping.

6. Place a maximum of 24 bags into the Bag Suspender. All nine trays are used regardless of the number of bags being processed. Place three bags per tray and then stack trays on center post with each level rotated 120 degrees. Insert the Bag Suspender with bags into the fiber analyzer vessel and place the Bag Suspender weight on top of the empty 9th tray to keep it submerged.

Note—Prior to inserting the Bag Suspender, if the vessel temperature is warm from a previous run, add cool water and exhaust.

7. When processing 24 sample bags, pour 1900-2000 mL of ambient temperature acid (0.255N H₂SO₄) solution into the fiber analyzer vessel. If

processing less than 20 bags, add 100 ml/bag of acid solution (use minimum of 1500 mL to ensure Bag Suspender is covered).

8. Turn Agitate and Heat on and confirm that Bag Suspender is agitating properly. Close and completely seal the lid of vessel. Extract samples for a total of 40 min.
9. At end of extraction, turn Heat and Agitate off. Open the drain valve (slowly at first) and exhaust hot solution before opening lid.

Note—The solution in the vessel is under pressure. The exhaust valve needs to be opened to release the pressure and solution prior to opening the lid.

10. After the solution has been exhausted, close the exhaust valve and open the lid. Add 1900 mL of (50-90°C) rinse water and agitate for 5 min. The lid may be sealed with the Heat on or left open with the Heat off. Repeat hot water rinse (total of two rinses).
11. When processing 24 sample bags, pour 1900-2000 mL of ambient temperature base (0.313N NaOH) solution over the Bag Suspender in the vessel. If processing less than 20 bags add 100 mL/bag of the base solution (minimum of 1500 mL).
12. Turn Agitate and Heat on and confirm that Bag Suspender is agitating properly. Close and completely seal the lid of vessel. Extract samples for a total of 40 min.
13. At end of extraction, turn Heat and Agitate off. Open the drain valve (slowly at first) and exhaust hot solution before opening lid.

Note—The solution in the vessel is under pressure. The exhaust valve needs to be opened to release the pressure and solution prior to opening the lid.

14. After the solution has been exhausted, close the exhaust valve and open the lid. Add 1900 mL of (50-90°C) rinse water and agitate for 5 min. The lid may be sealed with the Heat on or left open with the Heat off. Repeat hot water rinse (total of three rinses).
15. When the rinsing process is complete remove the samples. Gently press out excess water from bags. Place bags in a 250 mL beaker and add enough acetone to cover bags and soak for 3-5 min.
16. Remove bags from acetone and place on a wire screen to air-dry. Completely dry in oven at 102±2°C (most ovens will complete drying within 2-4 hrs).

Note—Do not place bags in the oven until acetone has completely evaporated.

17. Remove bags from oven, place directly into a collapsible desiccant pouch and flatten to remove air. Cool to ambient temperature and weigh bags.

Note—Do not use conventional desiccator container.

18. Ash the entire bag/sample in pre-weighed crucible for 2 hrs at 600±15°C, cool in desiccator and weigh to calculate loss of weight of organic matter (W₃).

CALCULATIONS

$$\% \text{ Crude Fiber} = 100 \times \frac{(W_3 - (W_1 \times C_1))}{W_2}$$

Where: W₁ = Bag tare weight

W₂ = Sample weight

W₃ = Weight of Organic Matter (Loss of weight on ignition of bag and fiber)

C₁ = Ash corrected blank bag factor (running average of loss of weight on ignition of blank bag/original blank bag)

PRECISION

Results of the collaborative study (see tables 1 & 2) indicates the precision (S_r, RSD_r, r) that the analyst should use as a benchmark for evaluating replication in the same laboratory.

NOTES

Caution

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.

Sodium hydroxide can severely burn skin, eyes and respiratory tract. Protective clothing should be worn when working with this acid. Always add caustic material to water and not the reverse.

Petroleum ether and acetone are extremely flammable. Avoid static electricity. A fume hood should be used at all times when using petroleum ether or acetone.

NUMBERED NOTES

1. F57 filter bags may produce up to 0.5% units lower values on finely ground feed samples. Finely ground samples are samples with fiber particles less than 25 microns.

2. A running average blank bag correction factor (C₁) should be used in the calculation of fiber. The inclusion of a blank bag in each run is mainly used as an indicator of particle loss. A C₁ 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 then grinding method needs to be evaluated.

Table 1. Results of the international collaborative study of the Filter Bag Technique for crude fiber compared with three laboratories using an Official Crude Fiber Method.

Collaborative Laboratory No.	Rep	Whole Corn	Cattle Feed	Alfalfa	Whole Soy	Poultry Starter	Calf Starter	Swine Feed	Horse Feed	Soy Meal	Pig Starter	Dog Food
% Crude Fiber												
1	1	2.1	14.5	22.6	9.8	4.7	11.0	17.5	6.4	3.7	2.8	1.3
	2	1.8	14.2	22.4	9.9	4.9	10.7	17.2	6.5	4.0	2.9	1.3
2	1	1.7	14.8 C	22.5	7.2 C	4.4	10.4	17.4	5.8	3.4	2.6	7.1 C
	2	2.0	20.2 C	23.0	10.1 C	4.7	11.1	17.4	6.0	3.5	2.8	1.0 C
3	1	1.6	14.1	22.5	10.1	4.6	10.8	17.6	6.6	3.9	3.1	2.0
	2	1.9	14.6	22.5	10.3	4.7	10.9	17.6	6.8	4.0	3.2	1.6
4	1	1.6	14.2	22.2	9.5	4.4	10.6	17.1	6.2	3.4	3.0	1.3
	2	1.7	14.7	22.2	9.9	4.7	10.5	16.9	6.4	3.7	2.9	1.3
5	1	1.5	13.9	22.7	9.5	4.8	10.5	17.3	5.9	3.6	2.8	1.3
	2	1.8	14.5	22.4	10.1	4.7	10.5	17.6	6.0	3.5	2.7	1.4
6	1	1.8	14.1	22.6	9.3	4.7	10.9	17.2	6.3	3.7	2.8	1.2
	2	2.0	14.3	21.9	9.4	4.5	10.4	17.2	6.1	3.8	3.0	1.3
7	1	1.7	14.5	24.0	10.0	4.8	10.7	17.4	6.1	3.7	3.0	1.2
	2	1.5	14.8	23.6	10.0	4.3	10.4	17.4	6.2	4.0	2.9	1.1
8	1	1.6	15.0	22.3	9.3	4.6	10.7	17.4 C	6.0 C	3.7	2.5	0.5
	2	1.6	14.4	22.9	10.0	4.3	10.8	2.4 C	5.2 C	3.4	2.6	1.1
9	1	1.4	14.4	21.9	8.9	4.6	10.4	17.0	5.9	3.4	2.7	1.3
	2	1.8	14.3	22.6	9.6	4.2	10.4	16.6	5.9	3.7	2.7	1.2
10	1	1.7	14.1	21.4	9.3	4.5	10.8	17.0	6.3	3.8	2.9	1.4
	2	1.7	14.2	22.1	9.8	4.8	10.9	17.3	6.3	3.6	2.8	1.4
11	1	1.4	14.3	23.3	8.5	4.7	10.9	17.7 C	6.1	3.6	2.8	1.3
	2	1.5	15.9	24.1	8.9	5.5	11.9	19.1 C	6.2	4.2	2.9	0.6
Mean		1.69	14.44	22.62	9.60	4.65	10.73	17.27	6.21	3.70	2.83	1.25
Official Method Laboratories^a												
% Crude Fiber												
Central Analytical		1.8	14.5	23.0	10.2	4.4	9.3 G	14.7 G	6.8	2.9	1.9 G	3.4 G
Hahn Laboratories, Inc.		2.0	14.0	21.2	8.4	4.2	10.6	17.4	5.7	4.2	2.9	1.6
SDSU Olson Bio. Lab		2.4	14.2	23.8	10.1	4.6	10.8	17.4	6.8	4.1	2.8	1.3
Mean		2.05	14.23	22.67	9.57	4.40	10.70	17.40	6.43	3.73	2.85	1.45

Outliers: C-Chochran, G-Grubbs, DG-Double Grubbs

^a AOCS Official Method Ba 6-84, AOAC 962.09

Table 2. Summary of the statistical analysis of the Filter Bag Technique crude fiber collaborative study, including comparison with the Official Method.

Sample type	Whole Corn	Cattle Feed	Alfalfa	Whole Soy	Poultry Starter	Calf Starter	Swine Feed	Horse Feed	Soy Meal	Pig Starter	Dog Food
Number of laboratories	11	10	11	10	11	11	9	10	11	11	10
Number of replicates	22	20	22	20	22	22	18	20	22	22	20
Overall FBT mean	1.69	14.44	22.62	9.60	4.65	10.73	17.27	6.21	3.70	2.83	1.25
Official Method mean ^a	2.05	14.23	22.67	9.57	4.40	10.70	17.40	6.43	3.73	2.85	1.45
S _r	0.16	0.44	0.36	0.32	0.26	0.28	0.18	0.10	0.20	0.09	0.23
S _R	0.19	0.44	0.67	0.48	0.27	0.33	0.28	0.27	0.22	0.17	0.31
RSD _r , %	9.6	3.1	1.6	3.3	5.5	2.6	1.1	1.6	5.3	3.3	18.1
RSD _R , %	11.4	3.1	2.9	5.0	5.8	3.1	1.6	4.3	6.0	6.0	24.5
r	0.46	1.23	1.00	0.88	0.72	0.80	0.51	0.27	0.55	0.26	0.64
R	0.54	1.23	1.86	1.34	0.75	0.94	0.78	0.75	0.62	0.48	0.86
HORRAT VALUE	3.07	1.14	1.18	1.75	1.82	1.11	0.62	1.42	1.83	1.75	6.34

^a Official Method AOCS Ba 6-84/AOAC 962.09

Neutral Detergent Fiber in Feeds Filter Bag Technique

(For A200,A200I)

DEFINITION

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

SCOPE

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

APPARATUS

1. Analytical Balance—capable of weighing down to 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 10-25 psi. The instrument must also be capable of creating a similar flow around each sample to ensure uniformity of extraction (ANKOM²⁰⁰, 65 rpm 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 rapid solution penetration (F57, ANKOM Technology).
5. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure (1915, ANKOM Technology).
6. Desiccator 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 30.0 g Sodium dodecyl sulfate, USP; 18.61g Ethylenediamine-tetraacetic disodium salt, dihydrate; 6.81 g Sodium borate; 4.56 g Sodium phosphate dibasic, anhydrous; and 10.0 ml Triethylene glycol, in 1 L distilled H₂O (premixed chemical solution available from ANKOM Technology). Check pH range to 6.9 to 7.1. Agitate and heat to aid solution. (see Notes, *Caution*).
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).

PREPARATION OF SAMPLE

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

PROCEDURE

1. Use a solvent resistant marker to label the filter bags. Weigh filter bag (W_1) and zero balance.
Note—Do not pre-dry filter bags; any moisture will be accounted for by the blank bag correction.
2. Weigh 0.45-0.55 g of prepared sample (W_2) directly in filter bag. Avoid placing the sample on the upper 4 mm of the bag.
3. Using a heat sealer, completely seal the upper edge of the filter bag within 4 mm of the top.
Note—Use sufficient heat to completely seal the filter bag and allow enough cool time (2 sec) before removing the bag from the heat sealer.
4. Weigh one blank bag and include in run to determine blank bag correction (C_1 , see Number Note 1).
5. **Pre-extract only samples containing soybean products or >5% fat:** Extract samples by placing 24 bags with samples into a container with a top. Pour enough acetone into container to cover bags and secure top. Shake the container 10 times and 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 container to cover bags and secure top. Shake the container 10 times and pour off acetone. Add fresh acetone and allow samples to soak for twelve hours. After soak time, pour out acetone and place bags on a wire screen to air-dry.
6. Place a maximum of 24 bags into the Bag Suspender. All nine trays should be used regardless of the number of bags being processed. Place three

bags per tray and then stack trays on center post with each level rotated 120 degrees. Insert the Bag Suspender with bags into the fiber analyzer vessel and place the weight on top to keep it submerged.

Note—Prior to inserting the Bag Suspender, if the vessel temperature is warm from a previous run, add cold water and exhaust.

7. When processing 24 sample bags, add 1900-2000mL of ambient ND solution to the fiber analyzer vessel. If processing less than 20 bags, add 100 ml/bag of ND solution (use minimum of 1500 mL to ensure Bag Suspender is covered). Add 20 g (0.5 g/50mL of ND solution) of sodium sulfite and 4.0 mL of alpha-amylase to the solution in the vessel.

8. Turn Agitate and Heat on and confirm agitation. Set timer for 75 min and close lid.

9. At end of extraction, turn Heat and Agitate off. Open the drain valve (slowly at first) and exhaust hot solution before opening lid.

Note—The solution in the vessel is under pressure. The exhaust valve needs to be opened to release the pressure and solution prior to opening the lid.

10. After the solution has been exhausted, close the exhaust valve and open the lid. Add 1900mL of (70-90°C) rinse water and 4.0 mL of alpha-amylase to the first and second rinses. Turn Agitate on and rinse for 5 min. The lid may be sealed with the Heat on or left open with the Heat off. Repeat hot water rinses a total of three times.

11. When the rinsing process is complete remove the samples. Gently press out excess water from bags. Place bags in a 250 mL beaker and add enough acetone to cover bags and soak for 3-5 min.

12. Remove bags from acetone and place on a wire screen to air-dry. Completely dry in oven at 102±2°C (most ovens will complete drying within 2-4 hrs).

Note—Do not place bags in the oven until acetone has completely evaporated.

13. Remove bags from oven, place directly into a collapsible desiccant pouch and flatten to remove air. Cool to ambient temperature and weigh bags (W₃).

Note—Do not use conventional desiccator container.

CALCULATIONS

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

Where: W₁ = Bag tare weight

W₂ = Sample weight

W₃ = Dried weight of bag with fiber after extraction process

C₁ = Blank bag correction (running average of final oven-dried weight divided by the original blank bag weight)

NOTES

Caution

Powdered chemicals will irritate the mucous membranes. A dust mask and gloves should be worn when handling this chemical.

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

NUMBERED NOTES

1. A running average blank bag correction factor (C₁) should be used in the calculation of fiber. The inclusion of a blank bag in each run is mainly used as an indicator of particle loss. A C₁ 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 then grinding method needs to be evaluated.