ANKOM 2000
FIBER ANALYZER

Operator's Manual

Rev E 1/14/15

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ANKOM TECHNOLOGY

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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 ANKOM^{2000} 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 product.

Warranty

ANKOM Technology warrants the ANKOM^{2000} 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. 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.

Extended warranties are available for purchase if desired.

Filter Bags

ANKOM Technology filter bags (part # F57) are designed to support precision and accuracy in analysis. 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 ANKOM^{2000} 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

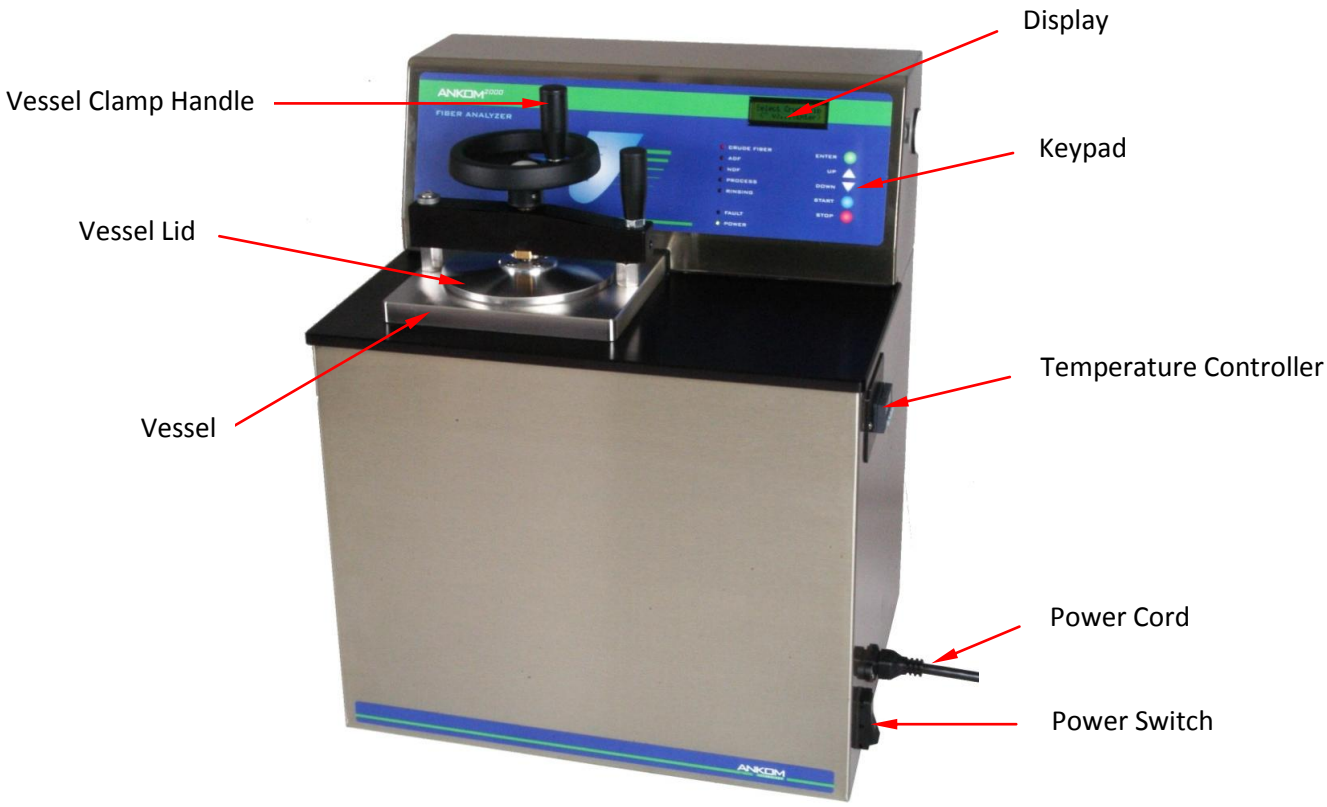
General Description

The ANKOM^{2000} 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.

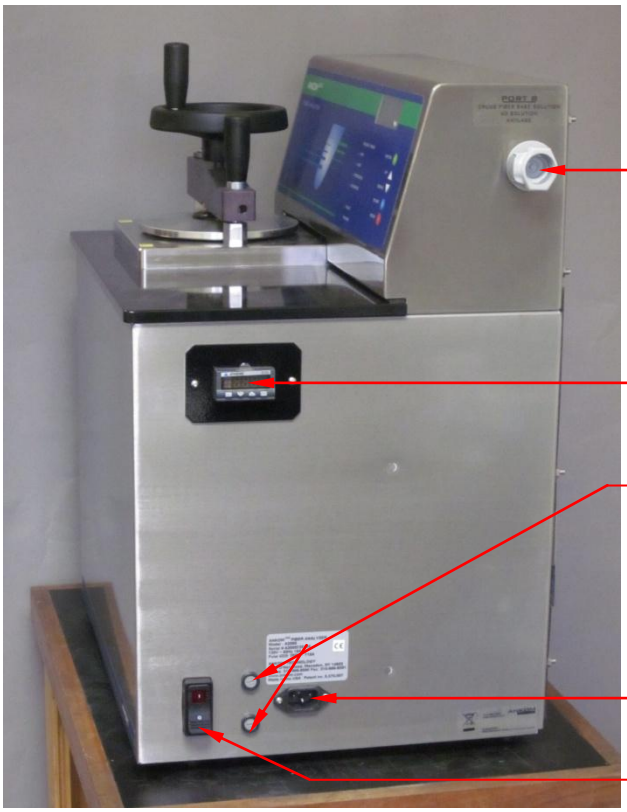
During analysis cell contents are removed as the encapsulated sample is subjected to the appropriate chemical (AD, ND, or crude fiber acid and base) solutions, leaving the desired fiber fraction. Results are determined gravimetrically. The filter bags are designed to allow proper flow of solutions while 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 ANKOM^{200} 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 ANKOM^{2000} Fiber Analyzer.

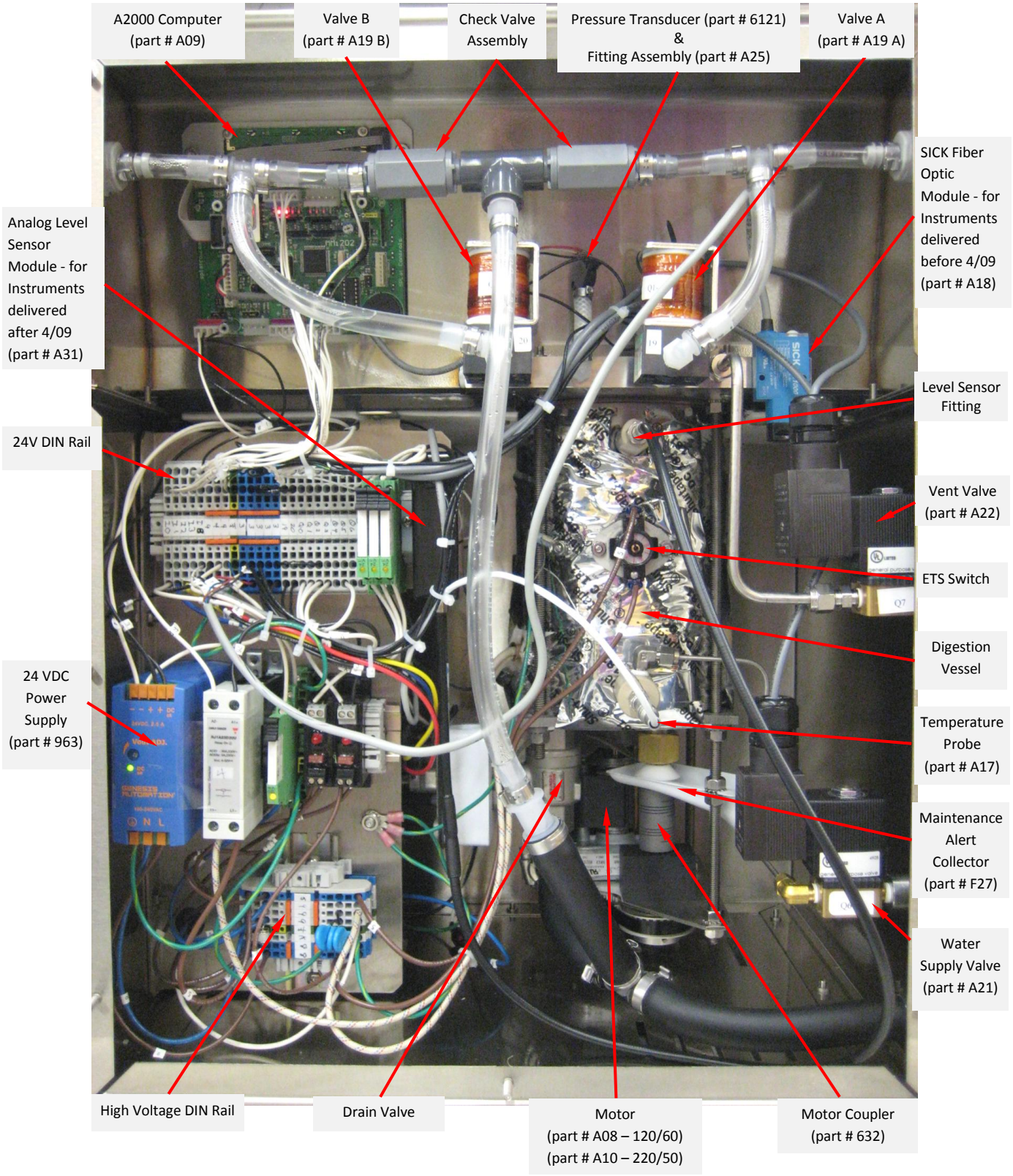


Left Side View



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 ANKOM^{2000} Fiber Analyzer you will need the following:

- Adjustable wrench
- Water supply located close to the ANKOM^{2000} 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 ANKOM^{2000} Fiber Analyzer, follow the procedure detailed below.

1. Remove the instrument from the shipping container and place it in an area that is within six 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.

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 Cubetainers for your chemicals, install the Cubetainer 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
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 ANKOM^{2000} Fiber Analyzer

The ANKOM^{2000} 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 ANKOM^{2000} also provides the capability to flush the solution lines.

The following sections provide the information you will need to use and maintain the ANKOM^{2000} Fiber Analyzer.

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ADF Analysis

ADF Calculation

ADF contained within a food or feed sample can be calculated 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)

ADF Sample Preparation Procedure

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

IMPORTANT

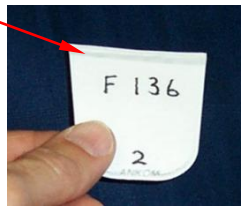
When using the ANKOM^{2000} Fiber Analyzer for ADF analysis, at least one blank filter bag should be included with the sample set as an indicator of particle loss. A running average of the blank bag weights is used in the fiber calculation as the C_1 correction factor. A C_1 value larger than 1.0000 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.

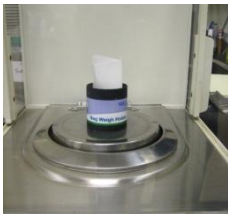
2. Weigh and record the weight of each empty filter bag (W_1).

3. Set the Heat Sealer dial to between 4 and 5. (The setting may vary from sealer to sealer.)

Seal



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.
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.)

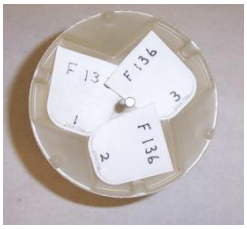
IMPORTANT

If your samples contain soybean products or >5% fat, before doing the ADF analysis in the ANKOM^{2000}, you will need to do a pre-extraction. For samples containing non-roasted soybean or >5% fat, follow the pre-extraction steps below:

1. Place the filter bags with sample (up to 23) into a container with a top.
2. Pour enough fresh acetone into the container to cover the bags.
3. Put the top on the container.
4. Shake the container 10 times and allow bags to soak for 10 minutes.
5. Pour out and dispose of the acetone.
6. Execute steps 1 through 5 a total of two times.
7. Place the bags on a wire screen to air-dry.

If your samples contain roasted soybean, follow the pre-extraction steps below:

1. Place the filter bags with sample (up to 23) into a container with a top.
2. Pour enough fresh acetone into the container to cover the bags.
3. Put the top on the container.
4. Shake the container 10 times.
5. Pour out and dispose of the acetone.
6. Pour fresh acetone into the container and allow the samples to soak for twelve hours.
7. Pour out the acetone.
8. Place the bags on a wire screen to air-dry.



12. 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).
13. 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.

14. 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 ANKOM^{2000} Fiber Analyzer

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

IMPORTANT

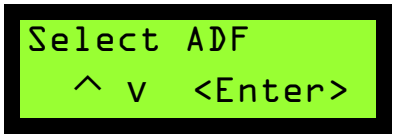
If you are changing from one analysis type to another, flush the system before running. See the "Flush Procedure" section of this manual for more information.

1. Verify that the hot water supply is on and the drain hose is securely positioned in the drain.
2. If you are using Cubetainers for your chemicals, attach the AD solution hose first to the Cubetainer 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.



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 Cubetainers to automatically add your solutions, you can manually fill the vessel for each procedure. First make sure that the hoses to the Cubetainers are disconnected. 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.

10. Press START on the Keypad. Solution from the Cubetainer 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 "Process Complete" message appears on the Display, the analyzer operation is complete. Open the Vessel Lid and remove the Samples at this time.
12. Gently press out excess water from the bags.
13. Place bags in a 250ml beaker and add enough acetone to cover them. Let the bags soak in acetone for 3 – 5 minutes.
14. Remove the bags from the acetone and place them on a wire screen to air-dry.

IMPORTANT

Do NOT place bags in the oven until the acetone has completely evaporated.

15. Place air-dried bags in the oven and heat at 102°C ± 2° for 2 – 4 hours (depending on the oven).
16. Remove the samples from the oven and place them in a Desiccant Pouch.

IMPORTANT

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

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

NDF Analysis

NDF Calculation

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

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

Where:

- W_1 = Bag tare weight
- W_2 = Sample weight
- W_3 = Dried weight of 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.

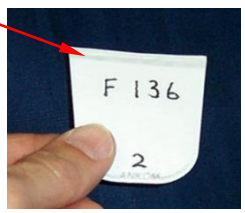
IMPORTANT

When using the ANKOM^{2000} 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(s). Any fiber particle loss from the filter bags will generate erroneous results. If particle loss is observed, the grinding method should be evaluated.

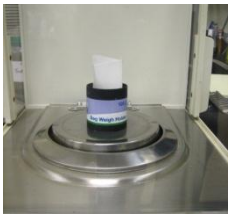
1. Using a Solvent Resistant Marker, number all of the filter bags you will use during the fiber analysis.

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.
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.)

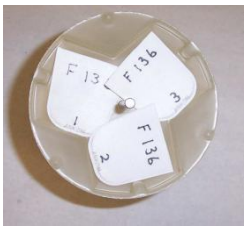
IMPORTANT

If your samples contain soybean products or >5% fat, before doing the NDF analysis in the ANKOM^{2000}, you will need to do a pre-extraction. For samples containing non-roasted soybean or >5% fat, follow the pre-extraction steps below:

1. Place the filter bags with sample (up to 23) into a container with a top.
2. Pour enough fresh acetone into the container to cover the bags.
3. Put the top on the container.
4. Shake the container 10 times and allow bags to soak for 10 minutes.
5. Pour out and dispose of the acetone.
6. Execute steps 1 through 5 a total of two times.
7. Place the bags on a wire screen to air-dry.

If your samples contain roasted soybean, follow the pre-extraction steps below:

1. Place the filter bags with sample (up to 23) into a container with a top.
2. Pour enough fresh acetone into the container to cover the bags.
3. Put the top on the container.
4. Shake the container 10 times.
5. Pour out and dispose of the acetone.
6. Pour fresh acetone into the container and allow the samples to soak for twelve hours.
7. Pour out the acetone.
8. Place the bags on a wire screen to air-dry.



12. 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).
13. 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.

14. 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.

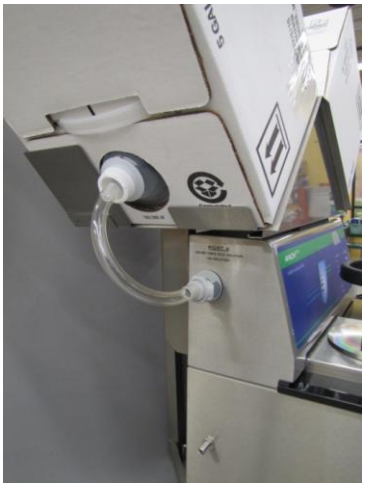
NDF Analysis Procedure using the ANKOM^{2000} Fiber Analyzer

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 Cubetainers for your chemicals, attach the ND solution hose first to the Cubetainer and then to Port A on the instrument.



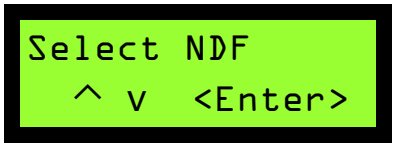
IMPORTANT

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. Fill half of the dispenser with water.
8. Add two capfuls (8ml) of amylase to the dispenser.
9. Fill the dispenser with water.

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.



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 Cubetainers to automatically add your solutions, you can manually fill the vessel for each procedure. First make sure that the hoses to the Cubetainers are disconnected. 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.

13. Press START on the Keypad. Solution from the Cubetainer 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 automatically inserted 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 "Process Complete" message appears on the Display, the analyzer operation is complete. Open the Vessel Lid and remove the Samples at this time.

17. Gently press out excess water from the bags.

18. Place bags in a 250ml beaker and add enough acetone to cover them. Let the bags soak in acetone for 3 – 5 minutes.

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

IMPORTANT

Do NOT place bags in the oven until the acetone has completely evaporated.

20. Place air-dried bags in the oven and heat at 102°C ± 2° for 2 – 4 hours (depending on the oven).
21. Remove the samples from the oven and place them in a Desiccant Pouch.

IMPORTANT

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

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

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:

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

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

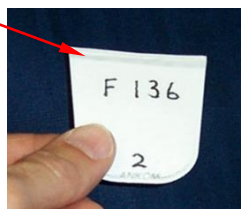
When using the ANKOM^{2000} Fiber Analyzer for Crude Fiber analysis, at least one blank filter bag should be included with the sample set as an indicator of particle loss. A running average of the blank bag weights is used in the fiber calculation as the C_1 correction factor. A C_1 value larger than 1.0000 indicates that sample particles were lost from filter bags and deposited on the blank bag(s). Any fiber particle loss from the filter bags will generate erroneous results. If particle loss is observed, the grinding method should be evaluated.

1. Using a Solvent Resistant Marker, number all of the filter bags you will use during the fiber analysis.

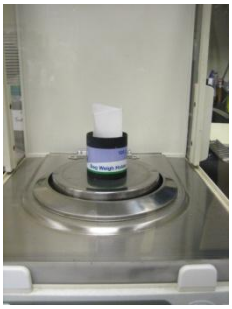
2. Weigh and record the weight of each empty filter bag (W_1).

3. Set the Heat Sealer dial to between 4 and 5. (The setting may vary from sealer to sealer.)

Seal



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.

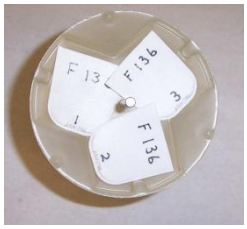


5. Place an empty filter bag in the Bag Holder in an open position.
6. Tare the weight of the empty filter bag and the holder together.
7. Add 0.95 – 1.00g of sample to the filter bag. Keep all particles away from the sealing area of the filter bag.
8. Record the weight of the sample (W_2) and tare.
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.)

IMPORTANT

For all samples you will need to do a pre-extraction of fat before doing a Crude Fiber analysis in the ANKOM*2000*. Follow the pre-extraction steps below:

1. Place the filter bags with sample into a 250ml container.
2. Pour enough petroleum ether into the container to cover the bags.
3. Allow the bags to soak for 10 minutes.
4. Pour out and dispose of the petroleum ether.
5. Place the bags on a wire screen to air-dry.



12. 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).
13. 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.

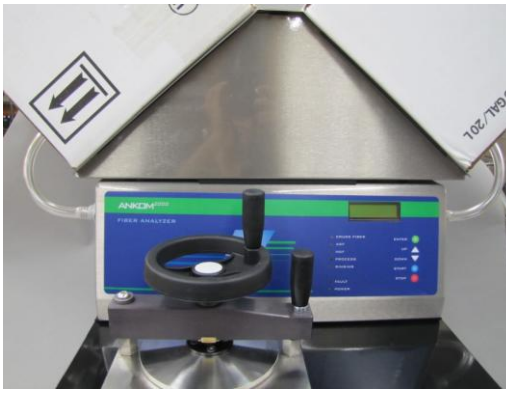
14. Add the 9th tray to the top of the Bag Suspender rod. This tray contains no filter bags and acts as a cover.

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

Crude Fiber Analysis Procedure using the ANKOM^{2000} Fiber Analyzer

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

1. Verify that the hot water supply is on and the drain hose is securely positioned in the drain.
2. Attach each Cubetainer hose first to the Cubetainer 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

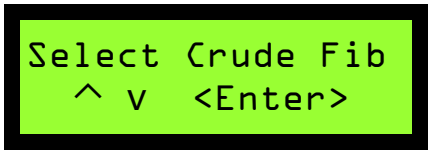
The solutions are gravity fed into the instrument ports. The bottom of the solution level must be at least 5" (13cm) above the port. The top of the solution level can be no more than 20" (50cm) above either port or the solution will overflow into the drain.

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.

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.

7. Press the arrow keys on the Keypad until you see "Select Crude Fib" on the Display.



IMPORTANT

The Crude Fiber analysis will use the following 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 "Process Complete" message appears on the Display, the analyzer operation is complete. Open the Vessel Lid and remove the Samples at this time.

12. Gently press out excess water from the bags.

13. Place bags in a 250ml beaker and add enough acetone to cover them. Let the bags soak in acetone for 3 – 5 minutes.

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

IMPORTANT

Do NOT place bags in the oven until the acetone has completely evaporated.

15. Place air-dried bags in the oven and heat at 102°C ± 2° for 2 – 4 hours (depending on the oven).

16. Remove the samples from the oven and place them in a Desiccant Pouch.

IMPORTANT

Do NOT use conventional countertop or cabinet desiccators for this part of the analysis.

17. Allow the samples to cool to room temperature. This should take about 10 – 15 minutes.
18. Re-weigh each filter bag immediately after removing from the Desiccant Pouch.
19. Ash all filter bags in pre-weighed crucibles for 2 hours at 600°C ± 15°.
20. Cool the ashed crucibles in a conventional desiccator.
21. Weigh the ashed crucibles to calculate the loss of weight of organic matter (W_3).

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Flush Procedure

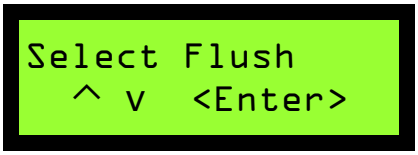
The Flush procedure allows you to clean the system with water. This procedure should be used when changing from one procedure to another (e.g., when preparing to do an ADF analysis after completing an NDF analysis) or when doing an NDF procedure after a previous NDF procedure with more than two hours 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 and follow the prompts on the Display to set up the instrument for the Flush procedure.
5. Attach the Amylase Dispenser Assembly to port B.
6. Fill the dispenser with hot water.
7. Attach the Amylase Dispenser Assembly to port A.
8. Fill the dispenser with hot water.

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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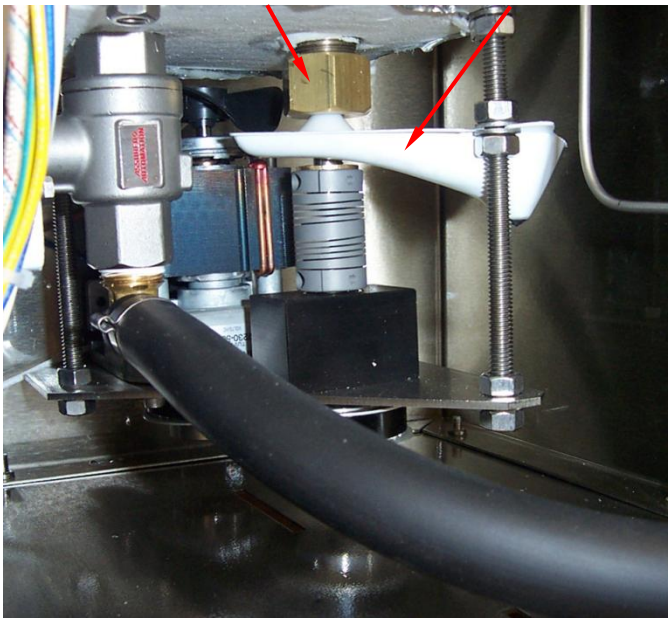
Periodic Maintenance

Initial Maintenance (after 10 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 Teflon washer has not come out of the filter body.

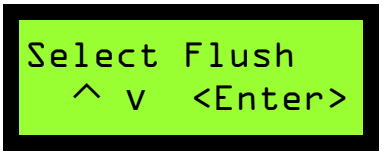


Rear View of instrument with cabinet back removed

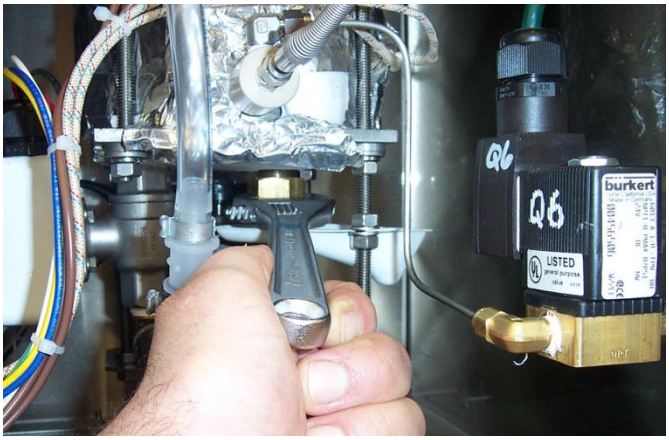
IMPORTANT

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.



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.



If you see a leak

If you see a leak, follow steps 6 – 9 in the Initial Maintenance procedure above.

Replacing the Fuses

To replace the fuses in the ANKOM*2000* 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)

Cleaning 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.

Check Agitation System and Bag Suspender

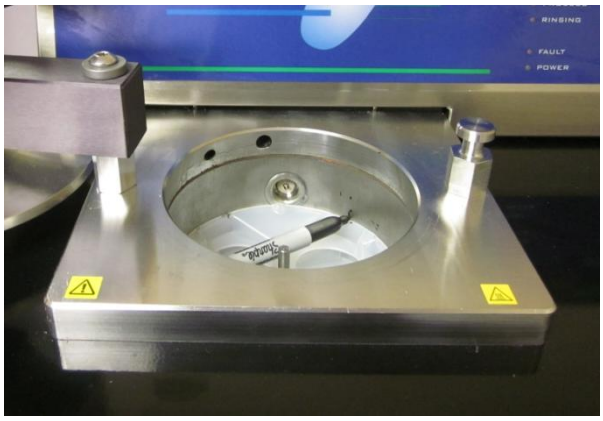
The agitation system and bag suspender should be checked every three to six months or if fiber values are higher than normal or inconsistent.

IMPORTANT

Poor agitation will cause higher analysis values and poor repeatability.

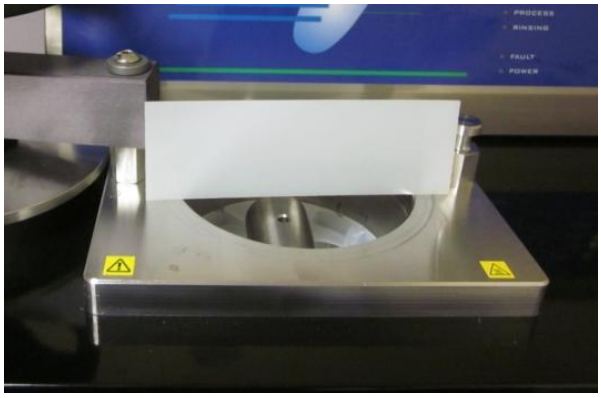
To check the stroke of the agitation system, follow the procedure detailed below.

1. Place a full bag suspender in the Vessel (without the bag suspender weight), but add NO water.
2. Remove the top from a dark felt tip marker.
3. Lay the marker horizontally on the top of the bag suspender so that the tip touches the inside wall of the Vessel.
4. With constant downward pressure on the marker, hold the pen in place so that it rides the top tray up and down as you execute the Flush Procedure. (See the Flush Procedure section of this manual for details.)
5. Allow the bag suspender (& pen) to move up and down three or four times as the pen marks the Vessel wall.
6. Stop the agitation.
7. Remove the pen and the bag suspender.
8. Measure the mark on the Vessel wall. It should be ½ inch long. If the motion is less than ½ inch, you will need to replace either the Bag Suspender Tip (see next page) or the agitator (because the old disc has flattened).



To test the agitator vertical positioning, follow the procedure detailed below.

1. Place the bag suspender weight on the bag suspender within the Vessel.
2. Place a straight edge across the top of the Vessel as shown in the picture.
3. Run the Flush Procedure. (See the Flush Procedure section of this manual for details.)
4. 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 re-seated at the bottom of the Vessel. Contact ANKOM for assistance.

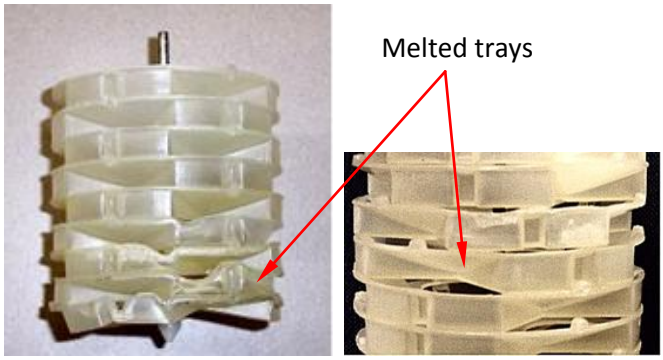


Check Bag Suspender

The bag suspender should be checked every three to six months or if fiber values are higher than normal or inconsistent.

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.



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.

Check the tip for excessive wear (see pictures).

Replace worn tips.

Troubleshooting

The ANKOM technology web site has the most current troubleshooting information. Therefore, if you have any questions about the operation of your ANKOM^{2000} Fiber Analyzer, please visit our web site at **www.ankom.com.**

Appendix A – ADF Method

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 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 ± 2°C (ANKOMRD Dryer, ANKOM Technology).
3. Digestion instrument—capable of performing the digestion at 100 ± 0.5°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 (ANKOM^{2000} 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, ANKOM Technology).
5. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure (1915, 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 H_2SO_4 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). See Numbered Notes 1.
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. **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 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.
   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 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.
7. Spread the sample uniformly inside the filter bags by shaking and flicking the bags to eliminate clumping.
8. 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.
9. Verify that the hot water supply is on and the drain hose is securely positioned in the drain.

(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)

Numbered Notes

1. 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.

ADF Procedure (continued)

10. Read the Temperature Controller on the right side of the instrument. If the temperature is higher than 20°C, cool the Vessel as follows:
    a. Fill the Vessel with cold water.
    b. When the Temperature Controller reads 20°C, run the Flush Procedure to drain the water.
11. If you are using Cubetainers for your chemicals, attach the AD solution hose to the Cubetainer and then to Port B on the instrument.
12. 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.
13. Follow the instructions on the ANKOM*2000* display:
    a. Select ADF.
    b. Close the Vessel Lid.
    c. Confirm hot water is on (>70°C).
    d. Press START.
14. 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.
15. 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.) NOTE: Do not place bags in the oven until the acetone has completely evaporated.
16. 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.

Appendix B – NDF Method

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 ± 2°C (ANKOMRD Dryer, ANKOM Technology).
3. Digestion instrument—capable of performing the digestion at 100 ± 0.5°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 (ANKOM^{2000} 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, ANKOM Technology).
5. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure (1915, 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 dodecyl 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 H_2O (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—Na_2SO_3, 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). See Numbered Notes 1.
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. **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 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.
   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 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.
7. Spread the sample uniformly inside the filter bags by shaking and flicking the bags to eliminate clumping.
8. 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.
9. Verify that the hot water supply is on and the drain hose is securely positioned in the drain.

(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)

Numbered Notes

1. 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.

NDF Procedure (continued)

10. If you are using Cubetainers for your chemicals, attach the ND solution hose to the Cubetainer and then to Port A on the instrument.
11. 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.
12. Follow the instructions on the ANKOM^{2000} display:
    a. Select NDF. (**Wait** to close the Vessel Lid.)
    b. Confirm hot water is on (>70°C).
    c. Press START.
    d. After the ND solution has been automatically inserted and agitation begins, manually add 20g of Na_2SO_3 and 4.0ml of alpha-amylase.
    e. Close the Vessel Lid.
13. 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 ANKOM^{2000} will automatically add the amylase solution to the first and second rinse.
14. 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.
15. 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.) NOTE: Do not place bags in the oven until the acetone has completely evaporated.
16. 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 C – Crude Fiber Method

Crude Fiber Analysis in Feeds - Filter Bag Technique (for A2000 and A2000I)

AOCS Approved Procedure Ba 6a-05

Definition

This method determines Crude Fiber which is the organic residue remaining after digesting with 0.255N H_2SO_4 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 0.1 mg.
2. Oven—capable of maintaining a temperature of 102 ± 2°C (ANKOMRD Dryer, ANKOM Technology).
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 10-25psi. The instrument must be capable of creating a similar flow around each sample to ensure uniformity of extraction (ANKOM^{2000} with 65rpm 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 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. Desiccant 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. 1.25g H_2SO_4/100ml. Concentration must be checked by titration.
   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.
2. Sodium hydroxide solution—0.3130 ± 005N. 1.25g NaOH/100ml. Concentration must be checked by titration.
   CAUTION2: Sodium hydroxide can severely burn the 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.

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.

Crude Fiber Procedure *(see the Crude Fiber 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 0.95 – 1.00g 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). See Numbered Notes 2.
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. Extract fat from samples by placing all bags into a 250ml container. Add enough petroleum ether to cover bags and soak for 10 minutes.

   CAUTION3: Petroleum ether is extremely flammable. Avoid static electricity. A fume hood should be used at all times when using petroleum ether.

   Pour off the solvent and allow the bags to air-dry. Spread the sample uniformly inside the filter bags by shaking and flicking the bags to eliminate clumping.
7. 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.
8. Verify that the hot water supply is on and the drain hose is securely positioned in the drain.
9. Read the Temperature Controller on the right side of the instrument. If the temperature is higher than 20°C, cool the Vessel as follows:
   a. Fill the Vessel with cold water.
   b. When the Temperature Controller reads 20°C, run the Flush Procedure to drain the water.

(Procedure continued on next page.)

Precision

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

Calculations

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

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)

Numbered Notes

1. F57 filter bags may produce up to 0.5% units lower values on finely ground samples. Finely ground samples have fiber particles less than 25 microns.
2. 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. 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.

Crude Fiber Procedure (continued)

10. Attach each Cubetainer hose to the Cubetainer and to its specific port. Port A is used for Crude Fiber Acid solution. Port B is used for Crude Fiber Base solution.
11. 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.
12. Follow the instructions on the ANKOM*2000* display:
    a. Select Crude Fiber.
    b. Close Vessel Lid.
    c. Confirm hot water is on (>50°C).
    d. Press START.
13. When the Crude Fiber extraction and rinsing processes are complete, open the Vessel Lid and remove the samples. Gently press out excess water from the bags. Place the bags in a 250ml beaker and add enough acetone to cover the bags and soak for 3-5 minutes.
    CAUTION4: Acetone is extremely flammable. Avoid static electricity. A fume hood should be used at all times when using acetone.
14. 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.) NOTE: Do not place bags in the oven until the acetone has completely evaporated.
15. 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. NOTE: Do not use a conventional desiccator container for this step.
16. Ash the entire filter bag/sample in a pre-weighed crucible for 2 hours at 600 ± 15°C, cool in a conventional desiccator and weigh to calculate loss of weight of organic matter (W_3).

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

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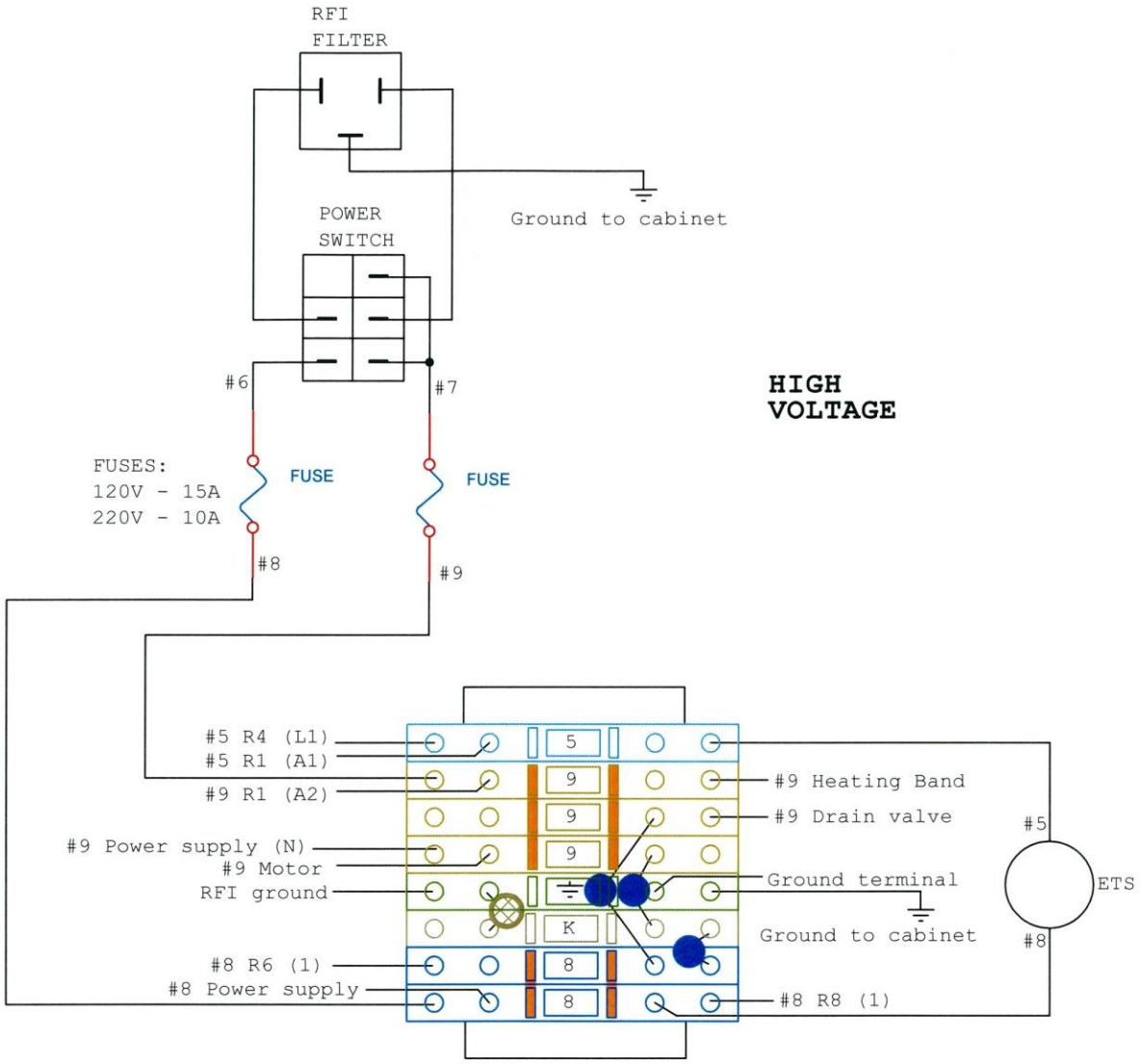
Appendix D – Parts & Assemblies

 **101.2** Bag Holder	 **X45** Desiccant Pouch	 **693** Bag Suspender Tray (Bottom)	 **694** Bag Suspender Tray (Top)
 **F11** Bag Suspender Assembly (with weight)	 **639.1** Bag Suspender Weight	 **F11.5** Kynar Tip w/ Washer Assembly for Bag Suspender	 **F12.5** Bag Suspender Rod Assembly
 **6044** Cubetainer w/ Tap	 **A04** Cubetainer Tube Assembly	 **A01** Shelf Assembly	 **A03** Amylase Dispenser Assembly
 **A06** External Drain Hose & Vent Tube Assembly	 **F12** External Drain Hose	 **657** Nylon Hose Clamp	 **714** Hose Coupler 2 pc

 **A07** Water Filter Assembly	 **A21** Water Valve Assembly	 **A22** Vent Valve Assembly	 **A19** B or A Valve Assembly (must specify B or A)
 **414** 24V Burkert Valve 1/8" Orifice	 **6121** Pressure Sensor	 **A25** Pressure Fitting Upgrade Assembly	 **Z132** Valve Seats
 **A02** Water Heater Assembly 120v	 **A30** Water Heater Assembly 220v	 **6032** Solid State Relay	 **6114** Solid State Relay
 **460** Relay 120v	 **461** Relay 220v	 **462** Relay 24V	 **6033** Relay Gold 24V
 **A09** Splat Controller Assembly	 **X90** Splat Display	 **A11** Controller Assembly	 **703** Filter RFI 220v

 **963** Power Supply	 **5653** On/Off Switch	 **687** Fuse 15A	 **6035** Fuse 10A
 **6151** SICK Fiber Optic Level Sensor Module (for instruments delivered before 4/09)	 **A18** SICK Optic Cable Assembly (for instruments delivered before 4/09)	 **6152** Fiber Optic Glass Tip 1/5"	 **A17** Temperature Probe Assembly
 **A31** Analog Optic Cable Assembly (for instruments delivered after 4/09)	 **6158** Analog Level Sensor Module (for instruments delivered after 4/09)	 **A08** Motor Assembly 120v	 **A10** Motor Assembly 220v
 **F8.9** Agitator Assembly with Peek Bushing	 **F28** Agitator Assembly w/ Packing Set	 **F26** Packing Set Assembly	 **632** Coupler
 **6068** Timing Belt	 **6058** O-Ring Viton 253	 **6041** Fan Blade	 **F27** Maintenance Alert Inner Assembly

Appendix E – Wiring Diagrams (1 of 4)



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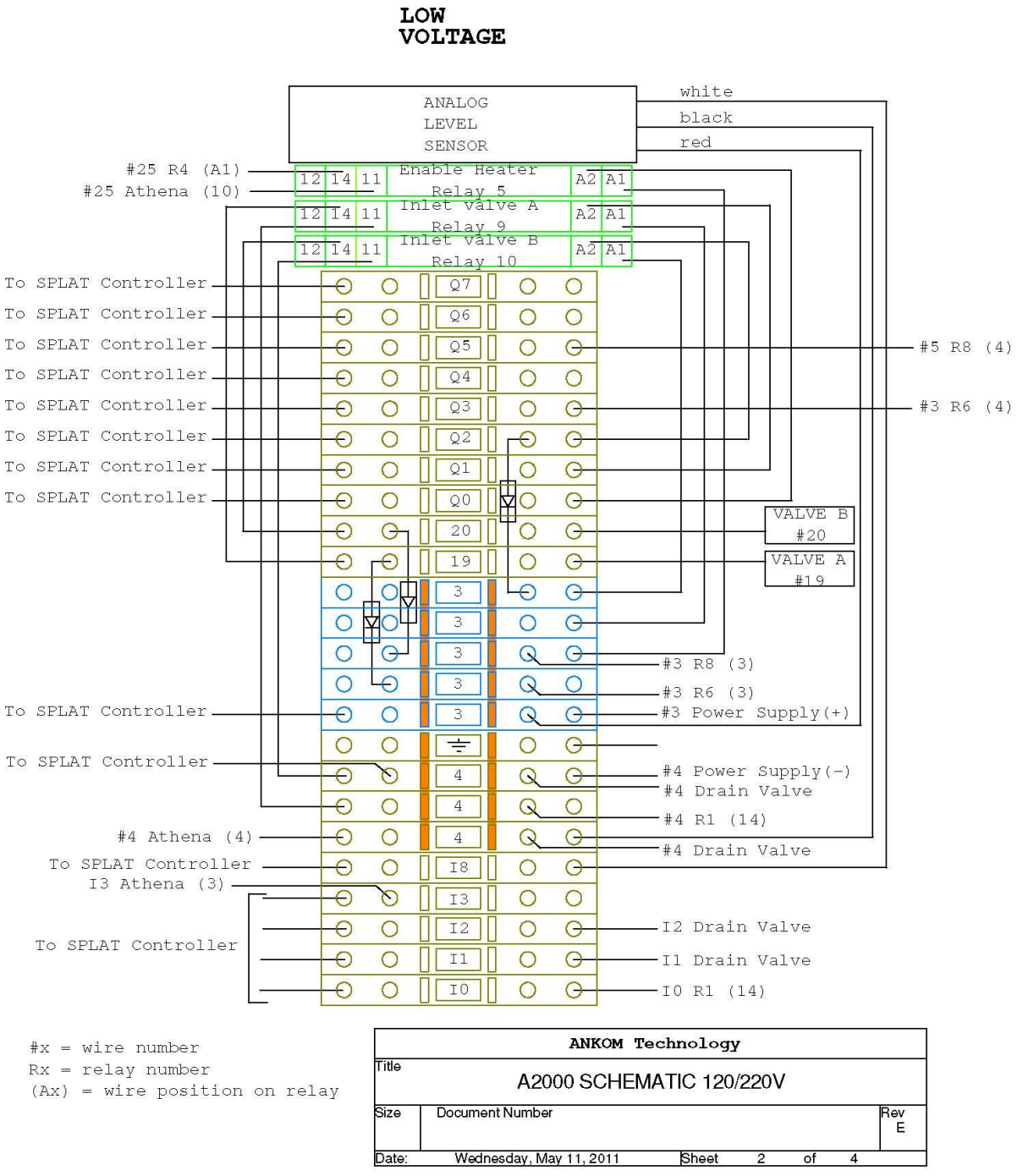
Date: Tuesday, September 21, 2010	Sheet 1 of 4		

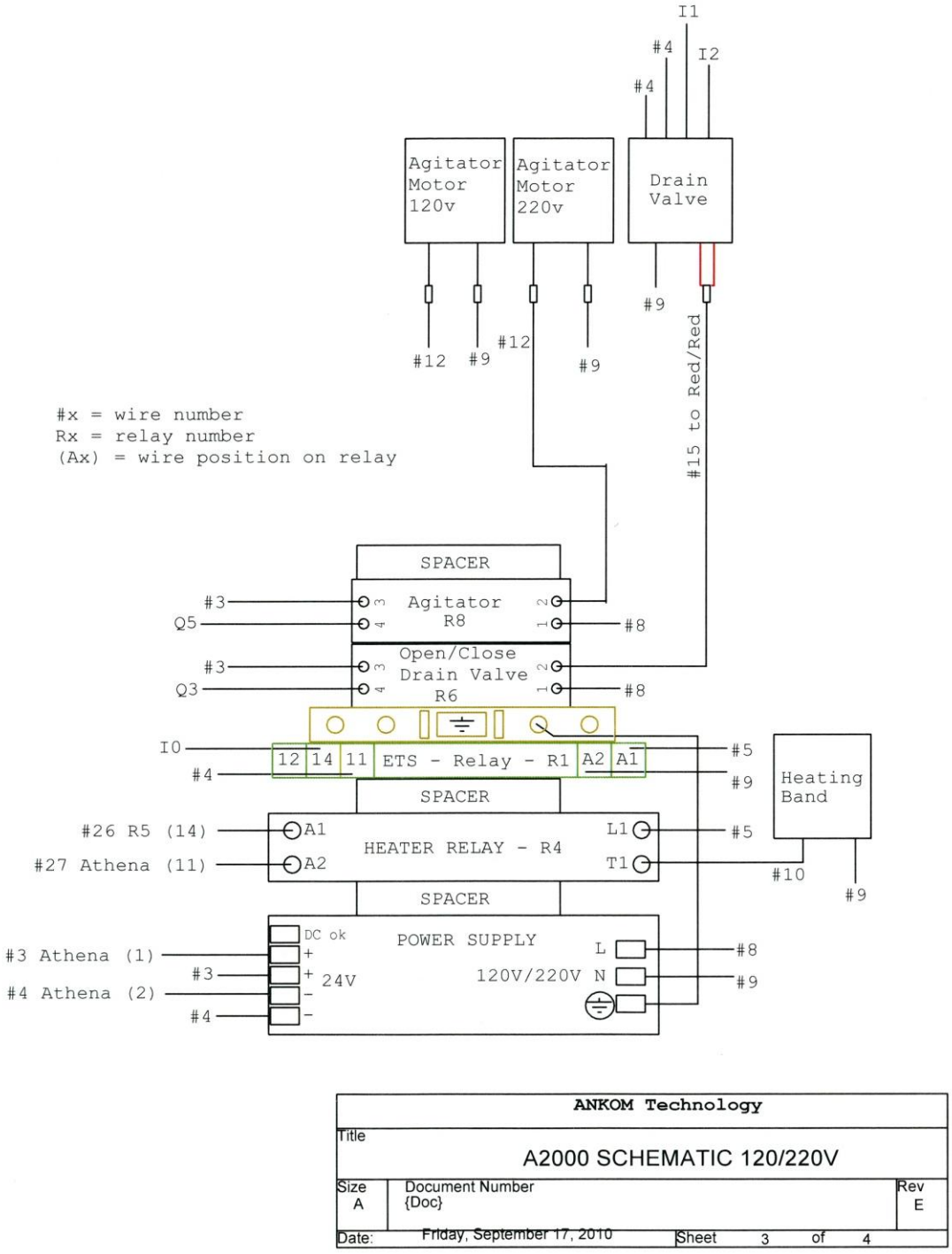
Appendix E – Wiring Diagrams (2 of 4)



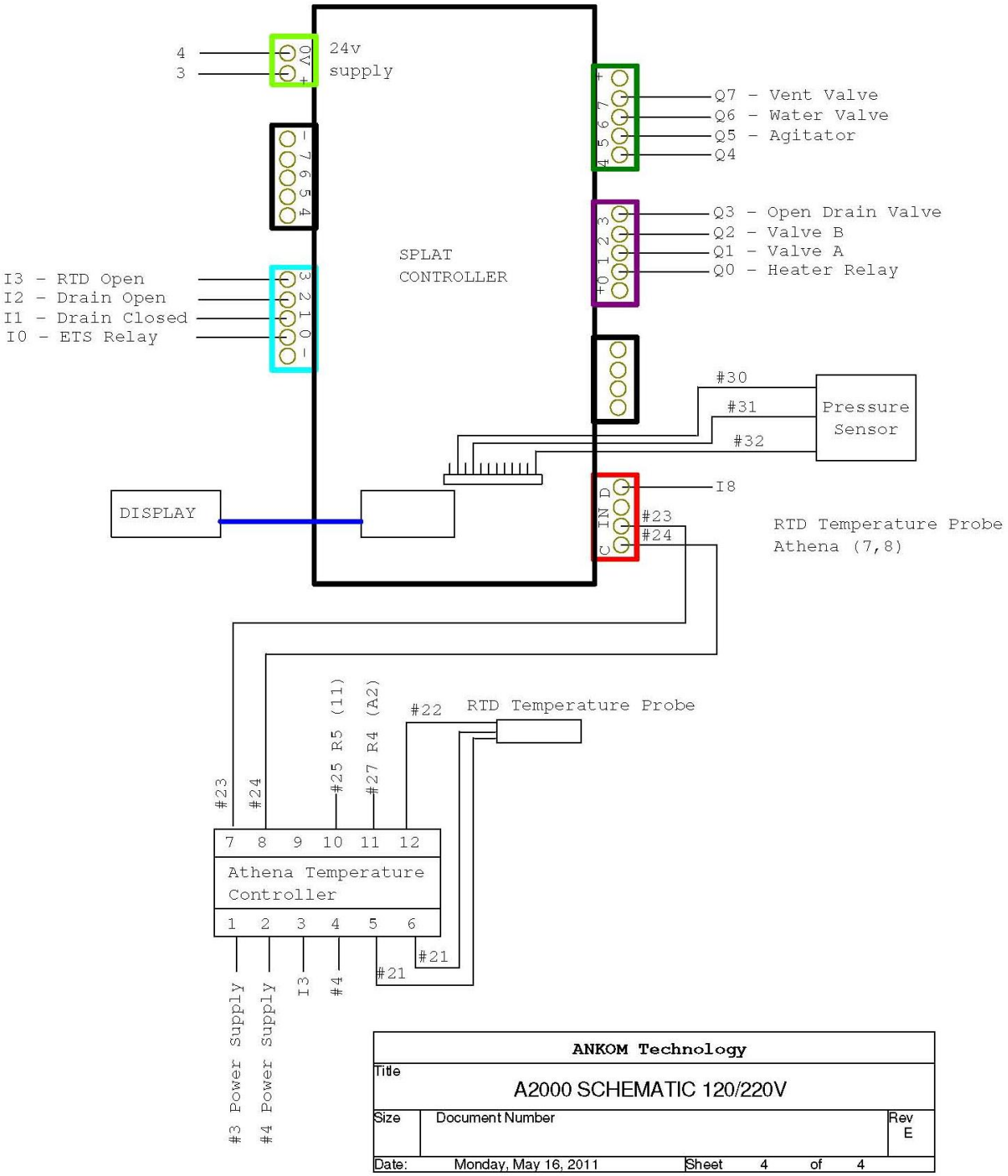
#x = wire number
Rx = relay number
(Ax) = wire position on relay

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

Appendix E – Wiring Diagrams (3 of 4)



Appendix E – Wiring Diagrams (4 of 4)



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