

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

### Definition

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

### Scope

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

### Apparatus

1. Analytical Balance—capable of weighing 0.1 mg.
2. Oven—capable of maintaining a temperature of  $102 \pm 2^\circ\text{C}$ .
3. Digestion instrument—capable of performing the digestion at  $100 \pm 0.5^\circ\text{C}$  and maintaining a pressure of 10-25psi. The instrument must be capable of creating a similar flow around each sample to ensure uniformity of extraction (ANKOM<sup>2000</sup> with 65rpm agitation, ANKOM Technology).
4. Filter Bags—constructed from chemically inert and heat resistant filter media, capable of being heat sealed closed and able to retain 25 micron particles while permitting solution penetration (F57 and F58, ANKOM Technology).
5. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure (HS or HSi, ANKOM Technology).
6. Desiccant Pouch—collapsible sealable pouch with desiccant inside that enables the removal of air from around the filter bags (*MoistureStop* weigh pouch, ANKOM Technology).
7. Marking pen—solvent and acid resistant (F08, ANKOM Technology).
4. Include at least one empty bag in the run to determine the blank bag correction ( $C_1$ ).  
NOTE: A running average blank bag correction factor ( $C_1$ ) should be used in the calculation of fiber. The inclusion of at least one blank bag in each run is mainly used as an indicator of particle loss. A  $C_1$  larger than 1.0000 indicates that sample particles were lost from filter bags and deposited on the blank bag during the extraction. Any fiber particle loss from the filter bags will generate erroneous results. If particle loss is observed then the grinding method needs to be evaluated.
5. Using a heat sealer, completely seal each filter bag closed within 4mm of the top to encapsulate the sample. NOTE: Use sufficient heat to completely seal the filter bags and allow enough cool time (2 sec) before removing each bag from the heat sealer.
6. **Pre-extract only samples containing >5% fat:** Extract samples by placing bags with samples into a container with a top. Pour enough acetone into the container to cover the bags and secure the top.  
**CAUTION2:** Acetone is extremely flammable. Avoid static electricity and use a fume hood when handling. 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.

### 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  $\text{H}_2\text{O}$  (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— $\text{Na}_2\text{SO}_3$ , anhydrous (FSS, ANKOM Technology)
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.

### 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.
9. Verify that the hot water supply is on and the drain hose is securely positioned in the drain.
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.

(Procedure continued on next page.)

**Calculations**

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

Where:

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

**NDF Procedure (continued)**

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 9<sup>th</sup> tray to keep the Bag Suspender submerged.

NOTE: If you do not want to use the Cubitainers to automatically add your solutions, you can manually fill the vessel for each procedure. First make sure that the hoses to the Cubitainers are disconnected. Follow steps 12a and 12b below. Then press START on the Keypad and immediately pour 2L of solution directly into the vessel. Then add 20g of Na<sub>2</sub>SO<sub>3</sub> and 4.0ml of alpha-amylase. Please note that the solution must cover the level sensor in order for the instrument to start its operation. Once agitation begins, close the Vessel Lid and follow the rest of the procedure from step 13 to the end.

12. Follow the instructions on the ANKOM<sup>2000</sup> 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<sub>2</sub>SO<sub>3</sub> 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<sup>2000</sup> 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.) Do not place bags in the oven until the acetone has completely evaporated.

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

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.