**Automated IDF/SDF Procedure**

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

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

### Apparatus
1. **Analytical Balance**—capable of weighing 0.1 mg.
2. **Drying Oven**—capable of maintaining a temperature of 105 ± 2°C.
3. **Fiber Recovery instrument** capable of separately recovering IDF and SDF residue. The instrument must be capable of automatically adding all reagents, mixing the sample to ensure proper digestion, and controlling digestion and precipitation temperatures (ANKOM\textsuperscript{DF} Dietary Fiber Analyzer, ANKOM Technology).
4. **Filter Bags** (DF-L, DF-S, ANKOM Technology).
5. **Bag Weigh Holder**—used for eliminating static during the bag weighing process (TDF52, ANKOM Technology).
6. **Drying Rack**—used for drying filter bags (TDF50, ANKOM Technology).
7. **Heat sealer**—sufficient for sealing the filter bags closed (HS, ANKOM Technology).
8. **Desiccant Pouch**—collapsible sealable pouch with desiccant inside that enables the removal of air from around the filter bags (MoistureStop weigh pouch, ANKOM Technology).
9. **Marking pen**—solvent and acid resistant (F08, ANKOM Technology).
10. **Acetone rinse stand** (TDF51 Rinse Stand, ANKOM Technology).
11. **Ashing Oven**.
12. **Protein Determination equipment**—Kjeldahl recommended.

### Reagents
Use Distilled Water throughout.

(a) **Ethanol 95%**.
(b) **Ethanol 78%**—Place 821 ml 95% ethanol into 1 L volumetric flask, dilute to volume with H\textsubscript{2}O. Thaw at 0°C.
(c) **Enzyme solutions**—To make your enzyme solutions for use in the ANKOM\textsuperscript{DF} Dietary Fiber Analyzer, dilute ANKOM Concentrate enzymes (TDF80/TDF81, TDF82/TDF83, TDF84/TDF85) with H\textsubscript{2}O according to the Enzyme Dilution Table below.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Amylase</td>
<td>Dilute 5 ml of amylase to 25 ml</td>
</tr>
<tr>
<td>Protease</td>
<td>Dilute 5 ml of protease to 25 ml</td>
</tr>
<tr>
<td>Amyloglucosidase</td>
<td>Dilute 5 ml of Amyloglucosidase to 25 ml</td>
</tr>
</tbody>
</table>

(d) **Diatomaceous earth (DE)**—(ANKOM DE1, DE2, or equivalent).
(e) **Acetone**—reagent grade.
(f) **MES**—2-(N-Morpholino)ethanesulfonic acid (MES, ANKOM Technology, or equivalent).
(g) **TRIS**—Tris(hydroxymethyl)aminomethane (TRIS, ANKOM Technology, or equivalent).
(h) **MES-TRIS buffer solution**—0.05M MES, 0.05M TRIS, pH 8.2 at 24°C. Dissolve 19.52 g MES and 12.2 g TRIS in 1.7 L H\textsubscript{2}O. Adjust pH to 8.2 with 6N NaOH, and dilute to 2 L with H\textsubscript{2}O. (Note: It is important to adjust pH to 8.2 at 24°C. However, if buffer temperature is 20°C, adjust pH to 8.3; if temperature is 28°C, adjust pH to 8.1. For deviations between 20 and 28°C, adjust by interpolation.)
(i) **Hydrochloric acid solution**—0.561N. Dilute 93.5 ml of 6N HCl to 1 L with H\textsubscript{2}O.

### Sample Preparation
1. Grind samples in a centrifugal mill with a 0.5 mm screen. Samples ground finer may have particle loss from the filter bags and result in low values.
2. De-fat and de-sugar samples as needed based on the AOAC 991.43, AACC 32-07.01, or NMKL 129,2003 methods. Adjust sample weights accordingly.

### IDF/SDF Procedure (see the IDF/SDF Analysis section of the Operator’s Manual for more detail)
1. Label the filter bags using a solvent resistant marker.
2. Fill chemical containers to the Min. Level line or above.
3. Fill all enzyme containers to the 15 ml line or above.
4. Place each filter bag in a tared Bag Weigh Holder and record the weight.
5. Place ca 1 g of DE in each of six tared and numbered tins and record the weights.
6. Place 0.5±0.05 g of sample in each of six tared and numbered tins and record the weights.
7. Remove all Clamp Bars from the instrument.
8. Follow the instructions on the Touch Screen Display (as detailed in steps 9-25 below).
9. Install SDF bags by gently pulling the black SDF Delivery Nozzle toward you and pulling each bag up so that the nozzle is inside the bag. Pull the bag up so that the top of the bag is about 35 mm (1.375 inches) above the top of Clamp Bar C and return the nozzle to its original position to hold the bag in place. Center each bag within the black lines located on the back of Clamp Bar C.
10. Re-install Clamp Bar D.
11. Flatten the bags to remove any wrinkles.
12. Press the check mark button ([\text{2}]) on the “SDF Bags (and clamp bar D) installed?” screen on the Touch Screen Display. This will close bar D which will pinch the bags just above the filter.
13. Add DE to each SDF bag, rinsing the tin with no more than 3 ml of Distilled Water to ensure complete DE transfer.
14. Install IDF bags by gently pulling the black IDF Delivery Nozzle toward you and pulling each bag up underneath the nozzle. Pull the bag up so that the top of the filter part of the IDF bag is just below the bottom of Clamp Bar B and return the nozzle to its original position to hold the bag in place. Center each bag within the black lines located on the back of Clamp Bar A.
15. Place at least 20 mm (0.75 inches) of each IDF bag filter inside the top of each corresponding SDF bag.
16. Re-install Clamp Bar B.
17. Flatten the bags to remove any wrinkles.
18. Press the check mark button ([\text{2}]) on the “IDF Bags (and clamp bar B) installed?” screen on the Touch Screen Display. This will close bar B which will pinch the bags just above the filter.
19. Re-install Clamp Bar C.
20. Transfer the prepared samples into each of the IDF bags, keeping it below the IDF Delivery Nozzle.
### Automated IDF/SDF Procedure

21. Secure the front of each SDF filter bag in place with the hook located on the front part of Clamp Bar C.
22. Check that the Nitrogen supply is connected to the instrument and turned on.
23. Set Filter Times.
24. Set the manual pH check.
25. Press the START button to begin the automated processes.
26. Check the pH during the IDF process, after the HCl has been added. (If configured, the instrument will stop so you can make this check.) Adjust to 4.0-4.7 as needed.
27. After the automated processes are complete, rinse the IDF and SDF bags twice with acetone. ANKOM recommends the use of the ANKOM TDF51 Rinse Stand for the acetone rinses.
28. After the acetone has evaporated, with your Heat Sealer set between 3 and 4 (settings may vary depending on the heat sealer and the power source), press the Heat Sealer arm down for 3 to 4 seconds to seal each bag just above the filter. This keeps all residue contained to the filter area while handling the bags.
29. Place each bag in the Drying Rack and place the rack in an oven set to 105°C. Dry to constant weight (about 90 min).
30. Remove all of the bags from the oven and place them in desiccant pouches to cool.
31. Removing only one filter bag from the desiccant pouches at a time, place each filter bag in a tared Bag Weigh Holder and record the weights.
32. Determine the protein content within the IDF residue. See the “Protein Determination Procedure – IDF” for more information.
33. Determine the protein content within the SDF residue. See the “Protein Determination Procedure – IDF / SDF / TDF” for more information.
34. Determine the ash content within the IDF and SDF residue. See the “Ash Determination Procedure – IDF / SDF / TDF” for more information.
35. Calculate % IDF and % SDF values.
36. Calculate % TDF by adding the % IDF and % SDF values.

### Calculations (all weights in grams)

#### % IDF

\[
\frac{[R_1 + R_2]/2 - P - A - B}{(M_1 + M_2)/2} \times 100
\]

Where:
- \(M_1, M_2\) = Original wt for duplicate samples adjusted for pre-treatment fat and sugar losses (g)
- \(R_1, R_2\) = Residue for duplicate samples (g)
- \(f_f\) = Final Filter Bag (g)
- \(f_i\) = Initial Filter Bag (g)
- \(P\) = Protein of residue and bag (g)
- \(A\) = Ash of residue and bag (g)
- \(B\) = Blank (g)

#### % SDF

\[
\frac{[(R_1 + R_2)/2 - P - A - B]}{(M_1 + M_2)/2} \times 100
\]

Where:
- \(M_1, M_2\) = Original wt for duplicate samples adjusted for pre-treatment fat and sugar losses (g)
- \(R_1, R_2\) = Residue for duplicate samples (g)
- \(f_f\) = Final Filter Bag (g)
- \(f_i\) = Initial Filter Bag (g)
- \(D\) = Original wt of Diatomaceous Earth (g)
- \(P\) = Protein of residue and bag (g)
- \(A\) = Ash of residue and bag (g)
- \(B\) = Blank (g)

#### % TDF

\[
\% TDF = \% IDF + \% SDF
\]