

## ANKOM Method for Determining ADF

### Materials:

Digestion Apparatus – ANKOM Fiber Analyzer  
ANKOM F57 Filter bags  
Impulse bag sealer  
Desiccator  
Moisture stop weigh pouch

### Reagents:

#### **Acid Detergent Solutions (1 N H<sub>2</sub>SO<sub>4</sub>):**

Add 55.68 ml concentrated Sulfuric acid (98%) (VWR Cat # 2876-46, FW 98.08, CAS # 7664-93-9) and 40 g Hexadecyltrimethylammonium bromide (Alfa Aesar Cat # A15235, FW 364.46, CAS # 57-09-0) to about 500 ml dd water. Dissolve and bring to a volume of 2 liters with dd water. Allow to stand overnight.

**Acetone** (Fisher Cat # A18-20, FW 58.08, CAS # 67-64-1)

### Procedure:

1. Weigh filter bag, record weight, and tare balance.
2. Weight 0.5 g air-dried sample, ground to pass through a 1 mm screen, directly into filter bag. Weigh two blank bags and include in digestion to determine bag correction.
3. Seal the bags closed within 0.5 cm from the open edge using the heat sealer.
4. Spread sample uniformly inside the filter bag by shaking and lightly flicking the bag to eliminate clumping.
5. A maximum of 24 bags may be placed inside the bag suspender. All nine trays are used regardless of the number of bags being processed. Place three bags per trays and stack trays on center post with each level rotated 120 degrees. The weight is placed on top of the empty ninth tray to keep the bag suspender submerged.

**Note – Samples containing soy or 0.5% fat:** Extract fat from sample by placing 24 bags with samples into a 500 ml bottle with a top. Pour enough acetone into bottle to cover bags and secure top. Shake the container 10 times and allow bags to soak 10 minutes. Repeat with fresh acetone. Pour out acetone and place bags on a wire screen to air dry.

An exception to this procedure is roasted soy. Roasted soy contains special properties, which require a modification to this procedure. Place roasted soy samples into a 500 ml bottle with a top. Pour enough acetone into bottle to cover bags and secure top. Shake the container 10 times and pour off acetone. Add fresh acetone and allow bags to soak for 12 hours. After soak time, drain off acetone and air dry.

6. When processing 24 bags add 1,900-2,000 ml of ADF solution into ANKOM Fiber Analyzer vessel. If processing less than 20 bags add 100 ml/bag of ADF solution (minimum of 1500 ml).

7. Place bag suspender into the solution in the vessel. Turn Agitate and Heat ON, and confirm that the bag suspender is correctly agitating. Set the timer for 60 minutes and push Start. Close and seal the lid of vessel.
8. After 60 minutes, turn Agitate and Heat OFF, open the drain valve and exhaust hot solution before opening lid.
9. After solution is exhausted, close valve and open lid. Add approximately 2,000 ml hot water. Lower lid but do not tighten. Turn Agitate ON and leave Heat OFF. Each rinse should last 3-5 minutes. Exhaust water and repeat for a total of four rinses.
10. Remove filter bags from suspender and gently press out water. Place bags into a beaker and soak in acetone for 3 minutes. Remove bags and lightly press out acetone.
11. Spread bags out and allow acetone to evaporate. Complete drying in a 100 °C oven for at least 2 hours. Remove bags from oven, place in a moisture stop pouch. Cool in desiccator and weigh bags.

**Calculations:**

Calculate percent NDF (DM basis):

$$[W3 - (W1 \times C1)] \times 100 \div W2 \times DM$$

Key: W1 = Bag tare weight  
W2 = Sample weight  
W3 = Weight after extraction process  
C1 = Blank bag correction (final oven dried weight/original blank bag weight)