

Reagents

Sulfuric acid (72% by weight) – ANKOM Technology FSA72 or dilute reagent grade H₂SO₄ to a specific gravity of 1634 g/L at 20°C (24.00N) by adding 1200 g H₂SO₄ to 350 ml H₂O in a 1 L MCA volumetric flask with cooling. Standardize this solution to 1634 g/L at 20°C specific gravity by removing solution and adding H₂O or H₂SO₄ as required.

Safety Precautions (see SDS)



WARNINGS:

Acetone is highly flammable. Use fume hood when handling acetone and avoid inhaling or contact with skin. Ensure that all the acetone has evaporated before placing it in the oven.

Wear rubber gloves and face shield when handling sulfuric acid. Always add sulfuric acid to water. If acid contacts skin, wash with copious amounts of water.

Apparatus

- a) Filtration device – ANKOM Technology – F57 Filter Bags

IMPORTANT: F58 bags can NOT be used for this procedure.

- b) Impulse bag sealer – ANKOM Technology – HS Heat Sealer
 c) Desiccator – ANKOM Technology – Desiccant/MoistureStop pouch – X45
 d) ANKOM Technology – **Daisy^{II} Incubator**

Procedure

- 1) Grind the sample to pass through a 1 mm screen (2mm screen when using a cyclone mill).
- 2) Weigh each Filter Bag (W₁), record the weight, and tare the balance.
- 3) Add 0.5 g (±0.05 g) of air-dried (As Received) sample (W₂) directly into each Filter Bag.
- 4) Weigh and seal one (1) blank bag and include it in the digestion to determine the blank bag correction (C₁).
- 5) Seal the bags closed 4 mm from the open edge using the heat sealer.
- 6) Spread the sample uniformly inside each filter bag by flicking the bag to eliminate clumping.
- 7) Perform ADF determinations using Fiber Analyzer (See ADF Procedure. W₃, Weight of dried bag (including fiber), after ADF analysis).
- 8) After performing ADF determinations, place up to 24 dried bags with samples into a **Daisy^{II} Incubator** jar, placing half of the samples on one side of the plastic divider and half on the other side.
- 9) Add 500 ml of 72% H₂SO₄ to cover the bags.

IMPORTANT: Bags must be completely dry and at ambient temperature before adding concentrate acid. If moisture (even ambient moisture) is present in the bags, heat generated by the H₂SO₄ and H₂O reaction will adversely affect the results.

- 10) Place jars into the **Daisy^{II} Incubator**. Turn on only the Rotation switch (do NOT turn the Heat switch on). Leave the door of the **Daisy^{II} Incubator** open and allow the jars with bags to rotate for 3 hours.
- 11) After 3 hours pour off the H₂SO₄ and **rinse with tap water to remove all acid**.

IMPORTANT: If acid remains in the bags when they go into the oven, the samples will burn, resulting in values that are higher than they should be.

- 12) Repeat rinses until pH paper shows neutral color when touching the bags. Rinse with approximately 250 ml of acetone for 3 minutes to remove the water. **Handle the bags gently during rinsing. Fine lignin particles can exit the filter if not handled carefully.**
- 13) Dry the bags in an oven at 105°C for 2-4 hours.



WARNING – Do NOT place bags in an oven until all acetone has evaporated.

IMPORTANT:

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.

- 14) Remove the bags from the oven and place them directly into Desiccant/MoistureStop pouches and flatten to remove air. Cool to ambient temperature and weigh the bags (W₄).
- 15) Prepare each bag for the ash procedure.

15.1) Fold each bag from bottom to top. Because the bags are wider at the top than at the bottom, there will be a little extra material on each side after the first fold.

Extra Material



15.2) Fold each bag from right to left. The extra material now lines up on the left side.

Extra Material



15.3) Heat seal the bag at the location of the extra material.



- 16) Ash the bags in pre-weighed crucibles (30 or 50 ml) at 525°C for 3 hours or until C-free. Cool and calculate weight loss (W₅).
- 17) Calculate blank bag ash correction (C₃) using weight loss upon ignition of a blank bag sequentially run through ADF and lignin steps.
- 18) Calculate percent ADL_{AR}.

*If desired, ADL_{DM} can be calculated to express lignin content per dry matter (DM).

$$ADL_{AR} = \frac{(W_5 - (W_1 \times C_3)) \times 100}{W_2}$$

$$*ADL_{DM} = \frac{(W_5 - (W_1 \times C_3)) \times 100}{W_2 \times DM}$$

Where:

- W₁ = Tare weight of empty bag
- W₂ = Weight of sample (As Received)
- W₃ = Weight of dried bag and fiber residue post ADF analysis

- W₄ = Weight of dried bag after sulfuric acid treatment
W₅ = Weight loss after ashing (represents bag + lignin)
C₁ = Blank bag correction (ADF_{AR} analysis)
C₃ = Blank bag correction (ADL_{AR} analysis)
ADL_{AR} = Acid Detergent lignin (As Received)
*ADL_{DM} = Acid Detergent lignin (Dry Matter Basis) - Customer to calculate if desired.