

## **Acid Detergent Lignin Procedure**

### **Materials:**

Polypropylene digestion tubes and glass rods  
Buchner funnels  
Filtering flasks  
Aluminum weighing pan  
Filtering flasks  
Glass filter papers (5.5 cm)  
Desiccator

### **Reagents:**

#### **Sulfuric Acid (72% by weight) Solution:**

72% H<sub>2</sub>SO<sub>4</sub> (add 2451 g sulfuric acid (96%) (VWR Cat # 2876-46, FW 98.08, CAS # 7664-93-9) to 817 g dd H<sub>2</sub>O) standardized to specific gravity of 1.634 at 20 °C.

### **Procedure:**

1. Place paper with residue from ADF procedure into a labeled 125 ml polypropylene digestion tube. Push to bottom with glass stirring rod. Save ADF pan.
2. Wearing safety glasses and gloves add 40 ml 72% H<sub>2</sub>SO<sub>4</sub>. Use glass rod to break up sample. Stir well. Leave glass rod in tube.
3. Stir once every hour. Digest 4 hours.
4. Filter contents onto glass filter papers under light vacuum.
5. Rinse tube and glass rod onto filter with hot dd water. Wash residue until free of acid (at least 250 ml).
6. Carefully transfer filter from funnel into aluminum weighing pan. Dry 8 hours or overnight in drying oven (100 °C).
7. Remove from oven and cool in desiccator. Record weight.
8. Ash dried residue in muffle furnace 8 hours or overnight (470 °C).
9. Remove slightly cooled samples and cool in desiccator. Record weight of ashed residue.

**Calculations:**

	A	B	C	D	E	F	G	H
Sample	Sample Weight	DM	Before Ash	After Ash	% Lignin dmb	Lignin Mean	sd	% CV
#2	0.3387	0.9700	1.4628	1.4591	1.126	1.15	0.033	2.89
	0.3075	0.9700	1.4369	1.4334	1.173			

$$E = \left[ \frac{C - D}{A \times B} \right] \times 100$$

$$1.126 = \left[ \frac{1.4628 - 1.4591}{0.3387 \times 0.9700} \right] \times 100$$

To calculate F, G, and H see statistical analysis procedure.

**Reference**

Goering, H. K. and P. J. Van Soest. 1970. Forage fiber analyses. Agr. Handbook No 379, ARS, USDA.

Fiber (acid detergent) and Lignin (H<sub>2</sub>SO<sub>4</sub>) in Animal Feed, AOAC Official Method 973.18, Official Methods of Analysis, 18<sup>th</sup> Ed, Revision 3, 2010