

## POYLYETHYLENE GLYCOL

Updated September 2013

### I. Reference:

Ulyatte, M.J. 1964. The use of polyethylene glycol as a marker for measuring rumen water volume and the rate of flow of water from the rumen of grazing sheep. N. Zealand J. Agr. Res. 7:713.

### II. Personal Protective Equipment:

- A. Lab coat
- B. Safety glasses/goggles
- C. Designated peroxide handling gloves

### III. Reagents:

- A. 0.3 N Barium Dihydroxide  $\text{Ba}(\text{OH})_2$ 
  - 1. Add 47.3235 g  $\text{Ba}(\text{OH})_2$  per liter of solution
  - 2. Some of the  $\text{Ba}(\text{OH})_2$  may settle out after being put into solution
  - 3. DO NOT agitate prior to use
- B. 5% (w/v) Zinc Sulfate  $\text{ZnSO}_4$ 
  - 1. 50 grams  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  per liter of solution
- 2. 10% (w/v) Barium Dichloride  $\text{BaCl}_2$ 
  - a. 100 grams  $\text{BaCl}_2$  per liter of solution
- 3. 30% (w/v) trichloroacetic acid (TCA) ( $\text{C}_2\text{HCl}_3\text{O}_2$ ) + 5.0% (w/v)  $\text{BaCl}_2$ 
  - a. 300 grams TCA + 50 grams  $\text{BaCl}_2$  per liter of solution

### IV. Procedure:

- A. Centrifuge abomasal or rumen samples for 10 minutes at 600-800 x g to remove particulate matter.
- B. To a clean test tube add:
  - 1. 2 ml supernatant from step A
  - 2. 1 ml 0.3 N  $\text{Ba}(\text{OH})_2$
  - 3. 1 ml 5%  $\text{ZnSO}_4$
  - 4. .5 ml 10%  $\text{BaCl}_2$
  - 5. 5.5 ml dd $\text{H}_2\text{O}$
- C. Mix well and let stand for 5 minutes
- D. Centrifuge for 10 minutes at 600-800 x g
- E. To a clean test tube add:
  - 1. 1 ml supernatant from step D
  - 2. 4 ml dd $\text{H}_2\text{O}$
  - 3. 5 ml 30% TCA + 5.0%  $\text{BaCl}_2$
  - 4. Mix well – read at 525nm at exactly 20 minutes on SpectraMax

**NOTE: Be sure to all used reagents go into a waste jar at the end of the procedure.**

V. Standards:

3000, 2250, 1500, 750, 375, 187.5  $\mu\text{g/ml}$

**IMPORTANT:** Do not use the same standard solutions for more than a week.

VI. Calculations:

Calculate regression line of standards vs. O.D. and fit samples to regression line.