Purine Assay

Materials:

25 ml screw cap culture tubes
16 x 125 mm disposable glass tubes
Whatman #4 filter paper, 9.0 cm
Water bath
Funnels
Pipettes, 100-1,000 ul and 2 ml
Marbles (large enough to cover tops of 16 x 125 test tubes)
Vortex mixer

Reagents:

a) **70% Perchloric Acid** (EMD Cat # PX0396A-7, FW 100.46, CAS # 7601-90-3)

b) **0.2 M Ammonium Phosphate Solution** (23 g Ammonium phosphate monobasic (Fisher Cat # A685-500, FW 115.03, CAS # 7722-76-1) dissolved in 1 liter water)

c) **0.0285 M Ammonium Phosphate Solution** (dilute buffer) (143 ml/ liter 0.2 M Ammonium phosphate solution (See "b" above) or 3.2775 g (ammonium phosphate monobasic (Fisher Cat # A685-500, FW 115.03, CAS # 7722-76-1)/ 1 H_2O)

d) **0.4 M Silver nitrate** (6.9 g Silver nitrate (Allied Chemical Cat # 2179, FW 169.89, CAS #7761-88-8 or VWR Cat # VW6030-4, FW 169.87, CAS # 7761-88-8) dissolved in 100 ml dd water (pH 2.00))

e) **0.5 N Hydrochloric acid** (41.3 ml 37% HCl (J.T. Baker Cat # 9530-33, FW 36.46, CAS # 7647-01-0) / liter water)

f) **Deionized distilled water, pH 2.00** (adjust pH with H_2SO_4)

g) **RNA standard torula yeast** (Sigma Cat # R6625, CAS # 63231-63-0) (Store desiccated in freezer.)

Procedure:

1. Weigh samples in duplicate into 25 ml screw cap tubes. Weigh out 0.5 g for duodenal, Dacron bags, and 0.2 g for bacterial samples. Leave one tube in rack blank for standard.
2. When ready to begin assay, weigh out 0.2000 g torula yeast RNA (standard) in the empty tube.
3. Add 2.5 $HClO_3$ (70% Perchloric acid) and tightly cap tube. Vortex until sample is wet. Incubate in 90-95 °C water bath for 15 minutes, remove from bath, vortex again (the better the sample is broken up at this step, the easier it will be to work with later on), and place in water bath for 45 minutes.
4. Add 8.75 ml dilute buffer (0.0285 M $NH_4H_2PO_4$), vortex vigorously to break up any clumps of black charred mass, and add another 8.75 ml dilute buffer (total of 17.5 ml). Make sure no black clumps are sticking to the sides of the tube. Vortex and place tubes into 90-95 °C water bath for 10-15 minutes. Filter (by gravity) through Whatman #4 filter paper into 16 x 125 mm disposable glass culture tubes.

5. Transfer 0.5 ml filtrate to 16 x 125 mm tubes, add 0.5 ml silver nitrate (0.4 M), 9 ml buffer (0.2 M $NH_4H_2PO_4$), and allow to stand overnight in refrigerator.

Note: For Dacron bag samples, transfer 2 ml filtrate from samples, add 2 ml $AgNO_3$ and 6 ml buffer. Treat standard and samples the same as above (0.5 ml filtrate, 0.5 ml $AgNO_3$ and 9 ml buffer).

6. Centrifuge for 10 minutes at 1,000 x g and draw off and discard the supernatant. Disturb the pellet as little as possible during this step.
7. Wash pellet with 6 ml dd water (pH 2.0). Vortex and repeat step 6.
8. Add 10 ml 0.5 N HCl and vortex until thoroughly mixed.
9. Cover tube with marble and allow to incubate in 90-95 °C water bath for 30 minutes.
10. Bacterial samples ONLY – repeat step 6.
11. Do not disturb or resuspend the pellet.
12. Dilute standard as follows:

ml Standard	ml 0.5 N HCl	mg RNA
0.25	9.75	5
0.50	9.50	10
0.75	9.25	15
1.00	9.00	20

Note: For Dacron bag and bacterial samples, include additional standards.

For Dacron Bag Sample

ml Standard	ml 0.5 N HCl	mg RNA
0.0625	9.937	1.25
0.125	9.875	2.50

For Bacterial Samples

ml Standard	ml 0.5 N HCl	mg RNA
1.25	8.75	25
1.50	8.50	30

13. Vortex all standard tubes.
14. Using the UV light source on the Spectrophotometer, read absorbance of standards and samples at 260 nm. The blank used is 0.5 HCl. Read the clear liquid fraction of samples.

Calculations:

$$RNA\frac{mg}{g} = \frac{Predicted\ concentration \div Sample\ Wt. \times DM}{Dilution\ Factor}$$

Predicted Concentration = Calculated by regression equation from the standard curve
Dilution Factor = determined by the amount of filtrate pipetted in Step 5 (Example: 0.5 ml filtrate = dilution factor of 1, 2 ml filtrate = dilution factor of 4).

$$\%\ RNA = \left(\frac{RNA\frac{mg}{g}}{1{,}000}\right) \times 100$$

Reference: Zinn, R. A. and F. N. Owns. 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. Can J. Anim. Sci. 66:157-166.