DIAMINOPIMELIC ACID
Updated September 2013

I. Reference:

Czerkawski, J.W. 1974. Methods for determining 2-6- diaminopimelic acid and 2-aminoethylphosphonic acid in gut contents. J. Sci. Fd. Agric. 25:45.

II. Personal Protective Equipment:

A. Lab coat
B. Safety glasses/goggles
C. Latex gloves

III. Reagents:

A. 6 N Hydrochloric Acid (HCl)
1. Measure 500 ml distilled H_2O into 1L volumetric
2. q.s. to 1L with 12 N HCl

B. 0.1M Citrate Buffer pH 2.0
1. Add 21.01 g citric acid ($C_6H_8O_7$) to 1L volumetric
2. Adjust pH to 2.0
3. q.s. to 1L with dH_2O

C. 0.1M Citrate Buffer pH 3.4
1. Add 21.01 g citric acid to 1L volumetric
2. Add 4.9 g Sodium Hydroxide (NaOH)
3. Adjust pH to 3.4
4. q.s. to 1L with dH_2O

D. 0.05M Citrate Buffer pH 4.2
1. 10.51 g citric acid to 1L volumetric
2. Add 2.8 g NaOH
3. Adjust pH to 4.2
4. q.s. to 1L with dH_2O

E. 90% Acetic Acid
1. Measure 900 ml glacial acetic acid ($C_2H_4O_2$) into 1L volumetric
2. q.s. to 1L with dH_2O

F. Acid Ninhydrin
1. Add 25.0 g Ninhydrin to 1L volumetric
2. Add 600 ml glacial acetic acid
3. Add164 ml 85% o-phosphoric acid
4. q.s. to 1L with dH_2O

NOTE: Handle acids in designated fume hood

IV. Hydrolysis:

A. Weigh approximately 500 mg duodenal sample into hydrolysis bottle
B. Add 50 ml 6 N HCl
C. Stopper and cap, bubble nitrogen through for at least 20 minutes
D. Hydrolyze at 105°C for 17 hours
E. Cool, filter through many layers of acid trt glass wool, rinse bottle well with distilled water into drying flask on rotary evaporator
F. Dry down at 70°C, (set H_2O bath to 70°C), rinse with distilled water and dry, repeat at

second time

G. Reconstitute with 15 ml 0.1M pH 2.0 citrate buffer

H. Using membrane filters, 5 µm size, (1" diameter), attach 50 ml disposable plastic syringe—add 1 scoop charcoal

I. Pour sample into syringe and let stand 5 minutes with occasional shaking

J. Aspirate through filter into sample bottle. Refrigerate or freeze (freeze unless sample will be analyzed within 72 hrs)

V. Ion Exchange:

A. Resin preparation:

1. Use Dowex 50 x 8 100-200 mesh.
2. Wash with 3 volumes 0.5 N HCl, stir at least 30 minutes
3. Filter and wash with distilled water until neutral
4. Wash with 3 volumes of 0.5 N NaOH, stir at least 30 minutes
5. Filter and wash with distilled water until neutral
6. Rinse twice with 0.1M pH 2.0 citrate buffer
7. Store in buffer at 2-8°C

B. Columns:

1. Pipette resin into 1 x 10 cm columns and pass 125 ml 0.1M pH 2.0 citrate buffer through prior to use
2. Do not allow to stand with just buffer too long – mold will grow
3. Do not let columns run dry
4. Pipette 10 ml sample into reservoir and allow to drain on to column
5. Pipette 0.25, 0.50, 0.75 and 1.0 ml of standard on to 4 columns at this point
6. Elute and collect as with samples
7. Concentrations of final standards used in calculations are 2.5, 5.0, 7.5, 10.0 g/ml
8. Put exactly 85 ml 0.1M pH 3.4 citrate buffer through column
9. Add 50 ml 0.05 M pH 4.2 citrate buffer to column
10. Collect eluate in 50 ml volumetric flasks – refrigerate

VI. Standards:

A. Stock Standard 0.5 mg/ml DAP:

1. Dissolve 50 mg DAP in 100 ml of 0.1M pH 2.0 citrate buffer

VII. Calculation:

A. Prepare a standard curve with standards and to get g/ml of each sample

$$\frac{\text{g/ml in sample} \times 1.5 \times 50}{1000} \text{ sample wt in mg} \times 100 = \%\text{DAP}$$

$$1.5 = \text{dilution factor } \frac{\text{15 ml reconstituted volume}}{\text{10 ml put on column}}$$

50 = dilution factor 50 ml eluate/1000 = puts value in mg