PEPSIN-PANCREATIN INSOLUBLE NITROGEN
Updated September 2013


II. Personal Protective Equipment:
   A. Lab coat
   B. Safety glasses/goggles
   C. Latex gloves

III. Reagents:
   A. Pepsin-HCl Solution
      1. Add 8.25 ml concentrated HCl to ~ 800 ml distilled H₂O
      2. Add 100 mg Pepsin and stir gently to dissolve.
      3. q.s. to 1 liter.
   B. 0.2 N NaOH.
      1. Add 10.3 g Sodium Hydroxide (NaOH) pellets to 1000 ml volumetric.
      2. Add a small amount of water to dissolve.
      3. q.s. to 1 liter.
   C. Pancreatin Solution in 0.5 M Phosphate Buffer, pH 8.0.
      1. Add 3.66 g Sodium Phosphate (NaH₂PO₄) (monobasic)
      2. Add 126.94 g Sodium Phosphate (NaH₂PO₄ ⋅7H₂O) (dibasic)
      3. Add ~ 800 ml distilled H₂O.
      4. Stir until dissolved.
      5. Add 0.5335 g pancreatin and stir gently to dissolve
      6. q.s. to 1 liter.

IV. Procedure:
   A. In 50 ml in vitro tubes weigh out sample containing 15 mg N in triplicate
   B. Per tube add 15 ml of pepsin-HCl solution.
   C. Incubate 3 hours at 30°C.
   D. Neutralize with 7.5 ml 0.2 N NaOH per tube.
   E. Add 7.5 ml pancreatin solution.
   F. Incubate additional 24 hours at 39°C.
   G. Filter through Whatman #541 filter paper as soon as possible.
   H. Dry filter paper and run Kjeldahls on them.

V. Calculations:
   A. Calculate % pepsin-pancreatin insoluble N or as % of total sample N.