ADENOSINE TRIPHOSPHATASE (ATP) BASIC STAINING PROTOCOL

Solutions:

A. 0.1 M Sodium Barbital Solution (5.15 gm barbital powder + deionized H₂O 250 ml)

B. 0.18 M Calcium Chloride (2.65 g CaCl₂2H₂O + deionized water 100 ml)

C. 1 w/v Calcium Chloride (5 g CaCl₂2H₂O + deionized water 500 ml)

D. 2 w/v Cobalt Chloride (4 g CaCl₂6H₂O + deionized water 200 ml)

E. "10,2 ATP Preincubation" FRESH
   20.0 ml  0.1 M Sodium Barbital
   20.0 ml  0.18 M Calcium Chloride
   60.0 ml  deionized water
   Adjust pH to 10.2 just prior to use with a few drops of 0.1 N NaOH

F. ATP Incubating Solution FRESH
   180 mg  ATP powder (disodium salt, Sigma # A-5394)
   18 ml   0.1 M Sodium Barbital
   63 ml   deionized water
   9 ml    0.18 M Calcium Chloride
   Add the calcium chloride last to prevent precipitation of ATP
   Prepare just prior to use and adjust pH to 9.4 with a few drops of 1N NaOH

Staining Procedure:

1. Unfreeze the coverslips with the biopsy

2. Incubate into 10,2 ATP Preincubation solution 15 minutes at room temperature.

3. rinse with deionized water one time.

4. incubate into ATP Incubating Solution 15 minutes 37°C

5. Wash 1% Calcium Chloride 3 x 3 min

6. stain 2% Cobalt Chloride 10 minutes.

7. Wash with 0.1M Sodium Barbital 1:20 solution, 3 times

8. Wash with deionized H₂O 5 times.

9. Prepare 1 % v/v solution of ammonium sulfide stain for 10 - 20 seconds

10. Rinse with tap water in the fume hood with approximately 5 changes.

11. Store in deionized water until ready for dehydration.

12. Glycerin gelatin cover