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ADENOSINE MONOPHOSPHATE DEAMINASE (AMPDA) PROTOCOL

PRINCIPLE:

AMPDA staining makes use of the spontaneous reduction of tetrazolium salts to formazans by thiols. The use of dithiothreitol increases the rate of reaction. Potassium chloride retards formazan production but activates adenosine monophosphate deaminase which increases the specificity of the reaction. A weakly buffered solution at a pH of ~6 allows the hydrolysis of AMP to IMP and the release of ammonia at the enzyme site which catalyzes the tetrazolium reduction by the added thiol.

SPECIMEN REQUIRED:

Snap frozen human striated muscle.

Controls:

Negative controls: Immerse a section into almost boiling water for several minutes, or; Human heart muscle. Then proceed with the staining procedure.

METHOD:

Fixation: None. Use snap frozen tissue.
Technique: Cut 10 - 16 micron (12 µm) sections in cryostat from snap frozen biopsy. Attach one or more sections to a No. 1½, 22 mm square coverslip.

Equipment:

Ceramic staining rack - Thomas Scientific #8542-E40
Columbia staining dish - Thomas Scientific #8542-C12
Columbia staining dish(jar) - Thomas Scientific #8542-E30
Forceps Latex gloves

Reagents:

Acetone - Baxter #010-4, **FLAMMABLE**
Adenosine 5' monophosphate, disodium salt; Store < 0 ^{o}C, desiccated
deionized water
DL-Dithiothreitol (DTT), Sigma D9163 **Noxious odor** Store at 0 - 5 ^{o}C
Gelatin - 100 bloom - MP Biomedicals #960317; Store at room temp.
Glycerol -Sigma G 8773 **IRRITANT** Store at room temperature
Nitro blue tetrazolium (NBT) - Sigma N6876, Store at 0- 5 ^{o} C
Potassium chloride Store at room temperature.
Phenol - Fisher A931-1, **CAUSTIC,** Store at room temperature

Solutions:

I. KCl 3 M

II. ACETONE DESTAINING SOLUTIONS (30%, 60%, 90%)
These solutions need only be approximate

30 % ≅ ACETONE	10 ml
deionized water	20 ml
60 % ≅ ACETONE	20 ml
deionized water	10 ml
90 % ≅ ACETONE	30 ml

III. Aqueous Mounting Medium (glycerogel)

Gelatin (MP BIOMEDICALS, LLC #960317 - 100 bloom	4 g
Glycerol	25 ml
Phenol (**CAUSTIC !**)	0.5 ml
deionized water	21 ml

1. Dissolve gelatin in boiling water.
2. Cool, but do not allow to solidify.
3. Add phenol and glycerol.
4. Mix well.
5. **Allow air bubbles in mixture to dissipate before using!**

Staining Procedure:

1. Weigh 10 mg NBT into a 30 ml beaker.
2. Weigh and add to the same beaker 4 mg AMP.
3. Add 9.3 ml deionized water to beaker and dissolve.
4. Slowly pipet 0.7 ml 3M KCl to solution while it is stirring.
5. pH solution to 6.1 $\pm$ 0.03 with 1 $\rightarrow$ 2 drops of 0.1N NaOH **THIS CAN BE DIFFICULT. IF THE SOLUTION BECOMES TOO ALKALINE, IT WILL TURN BLUE AND YOU MUST START FROM THE BEGINNING.**
6. Weigh 7 mg DTT, add to already pH 'ed solution and mix well.
7. Incubate coverslips in a Columbia staining dish (Thomas Scientific #8542-C12) for 30 minutes at room temperature.
8. Wash with three exchanges of tap or deionized H_2O.
9. Prepare approximate solutions of 30, 60 and 90 % acetone using deionized H_2O and remove unbound NBT from the sections with three exchanges each of the acetone solutions in increasing then decreasing concentration. Leave the 90 % acetone covering the sections until a faint purplish cloud is seen over the section.
10. Rinse several times with deionized H_2O. Then mount the coverslips with the aqueous mounting medium onto a labeled glass slide.

Results:

Human type 1 fibers stain with a heavy blue stippling on a clear background. Type 2 fibers stain with a fine reticular pattern on a pink to purple background. Staining is essentially absent in negative control sections.

REFERENCES:

1. Fishbein, W., Fed. Proceed., 36 , 1061 (1977).
2. Fishbein, W., Fed. Proceed., 37 , 331 (1978).
3. Fishbein, W., Arch. Pathol. Lab. Med., 104, 462 (1980)