Washington University School of Medicine
Neuromuscular Lab
CAP: 1923316
CLIA: 26D0652044
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SUDAN BLACK B PROTOCOL

PRINCIPLE:

The Sudan black technique (modified Chiffelle & Putt) involves primarily physical processes in contrast to most staining procedures with chemical mechanisms. The dye is dissolved in a lipid solvent, and sections are treated with the dye-solvent solution. The dye is more soluble in the lipid in the tissue section than in the original solvent, the dye will move out of the solvent and color the tissue lipid. Boundary-surface adsorption plays a prominent role in the process. The staining is of a physical nature. Chemically different lipids cannot be distinguished by this method. The best fixatives are neutral buffered formalin or formal-calcium. Simple lipid stains that demonstrate the presence or absence of lipids must be performed on frozen sections. Propylene glycol is the dye solvent so the dye solvent will not dissolve out any of the tissue lipid. Aqueous mounting media, such as glycerin jelly is used to mount the finished slide because the organic solvents present in synthetic resins would dissolve the dye-lipid complex.

SPECIMEN REQUIRED:

Snap frozen human striated muscle.

QUALITY ASSURANCE:

Stain several different muscles simultaneously. Sudan black lipid is normally present in connective tissue. Droplets in muscle fibers are of pathologic interest.

METHOD:

Fixation: Use snap frozen tissue for sections. Fix sections before staining.

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:

Calcium Chloride, anhydrous - Sigma C4901, Store at room temperature
deionized water
Formaldehyde, 37 % - Fisher F79-500, **POISON, CARCINOGEN**,
Store at room temperature
Gelatin - 100 bloom -MP Biomedicals, LLC Cat # 960317 (via Fisher ?)
Glycerol -Sigma G 8773, store at room temperature **IRRITANT**
Phenol - Fisher A931-1, **CAUSTIC,** Store at room temperature
Propylene Glycol - Fisher P355-1, **HEALTH HAZARD**
EYES, CNS, TERATOGENIC, Store at room temperature
Sudan Black B - Fisher S-668, Store at room temperature

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Solutions:

I. BAKER'S FIXATIVE (modified calcium-formol)

Calcium Chloride, anhydrous ($CaCl_2$)		0.75 g
Formaldehyde, 37%(HCHO)		7.5 ml
deionized water	→	250 ml

Store at room temperature

II. Propylene Glycol, 85 % (v/v)

Propylene Glycol, 100 %	85 ml
deionized water	15 ml

Store at room temperature

III. Sudan Black B Staining Solution

Sudan Black B dye	0.7 g
Propylene Glycol, 100 %	100 ml

Dissolve dye in a small amount of propylene glycol in a 250 ml glass beaker with a magnetic stir bar and place on a hot plate/stirrer. Add small amounts of propylene glycol at a time with stirring and heat to ***Do Not Exceed 110 ^{o}C.***
Filter using vacuum with a small Buchner funnel and a coarse, fast filter paper while still warm. Cool to room temperature and filter through a fritted glass filter of medium porosity with vacuum.
Store at room temperature.

IV. Aqueous Mounting Medium (Glycerogel)

Gelatin (ICN#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. Add Baker's Fixative to coverslips with sections in a columbia staining dish (Thomas Scientific #8542-C12) for 5 minutes.

2. Wash with three exchanges of tap or deionized H_2O.

3. Add 100 % propylene glycol to dehydrate for 5 minutes.

4. Decant propylene glycol and add Sudan Black B Stain for a minimum of 2 hours (overnight is preferred).

5. Wash with three exchanges of tap or deionized H_2O.

6. Wipe the backs of the coverslips clean using cotton tipped swabs.

7. Differentiate in 85 % propylene glycol for 3 minutes.

8. Rinse several times with deionized H_2O and then gently mount the coverslips with the aqueous mounting medium onto a labeled glass slide.

9. If air bubbles are present after cover slipping, they should not be pressed out, since this process will displace stained lipid. Instead, re-immerse the slide in warm water until the coverslip falls off; re-wipe excess water from the slide and re-coverslip.

Results:

Fat deposits are blue-black. There is more staining of type I than II muscle fibers. Excess lipid is manifest by enlarged droplets in muscle fibers.

REFERENCES:

1. Sheehan, D.C. and Hrapchak, B.B.: **THEORY AND PRACTICE OF HISTOTECHNOLOGY** Second Edition, Battelle Memorial Institute, 1987.

2. Thompson, **SELECTED HISTOCHEMICAL AND HISTOPATHOLOGICAL METHODS**, Charles C. Thomas, Springfield, IL, 1966.