Subject:

KARNOVSKY'S GLUTARALDEHYDE

PURPOSE: A fixative for electron microscopy.

REAGENTS:

Stock Solution:

Paraformaldehyde	2.0 gm
1M Sodium hydroxide	2 - 4 drops
50% glutaraldehyde	5.0 ml (25% glut - 10 ml)
0.2M cacodylate buffer, pH7.4	20.0 ml

Mix the paraformaldehyde with 25 ml of distilled water in a 125 ml Erlenmyer flask. Heat to 60°C. on a stir plate. When moisture forms on the sides of flask, add sodium hydroxide and stir until the solution clears. Cool solution under the faucet. Filter, add glutaraldehyde and 0.2M buffer, pH range 7.2 to 7.4.

Sodium Cacodylate Buffers:

0.1M:

Sodium cacodylate	4.28 gm
Calcium chloride	25.0 gm
0.2N hydrochloric acid	2.5 ml

Dilute to 200 ml with distilled water, pH 7.4

0.2M:

Sodium cacodylate	8.56 gm

Mix as above, pH to 7.4 with HCL.

Working Solution:

Dilute 1:4 with 0.1M sodium cacodylate buffer to obtain an osmolarity of over 500 mOsm.

Dilute 1:2 for osmolarity over 700 mOsm.

Place in individual vials, label, date and freeze.

CAUTION: POSSIBLE CARCINOGEN

SAFETY: Use caution, work in a well ventilated area, wear gloves, lab coat, and goggles.

Paraformaldehyde: severe eye and skin irritant. Sensitizer by skin and respiratory contact. Toxic by ingestion and inhalation. Target organ effects on respiratory system. Corrosive. Carcinogen.

Gluteraldehyde: Severe skin and eye irritant; toxic by ingestion.

FIXATION TIME: 1 hour minimum, 2 to 3 hours preferred, can be left overnight

PROCEDURE:

1. Thaw vial of fixative.
2. Place tissue sample in fixative within 15 minutes of removal from patient.
3. Date and time tissue is placed in fixative.

REFERENCE: Sheehan,D and Hrapchak,B: Theory and practice of Histotechnology, 2nd Ed, 1980, pp 330-331, Battelle Press, Ohio

Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: By:

Approved: By:

KARNOVSKY FIXATIVE

Stock Solution:

Paraformaldehyde	2.0 gm
1M Sodium hydroxide	2 - 4 drops
50% glutaraldehyde	5.0 ml
(25% glut 10.0 ml)	
0.2M cacodylate buffer, pH7.4	20.0 ml

Mix the paraformaldehyde with 25 ml of distilled water in a 125 ml Erlenmyer flask. Heat to 60°C. on a stir plate. When moisture forms on the sides of flask, add sodium hydroxide and stir until the solution clears. Cool solution under the faucet. Filter, add glutaraldehyde and 0.2M buffer, pH range 7.2 to 7.4.

Sodium Cacodylate Buffers:

0.1M:

Sodium cacodylate	4.28 gm
Calcium chloride	25.0 gm
0.2N hydrochloric acid	2.5 ml

Dilute to 200 ml with distilled water, pH 7.4

0.2M:

Sodium cacodylate	8.56 gm

Mix as above, pH to 7.4 with HCL.

Working Solution:

Dilute 1:4 with 0.1M sodium cacodylate buffer to obtain an osmolarity of over 500 mOsm.
Dilute 1:2 for osmolarity over 700 mOsm.
Place in individual vials, label, date and freeze.

CAUTION: POSSIBLE CARCINOGEN

Obtain through VAH EM Laboratory and LDS EM Laboratory.

SAFETY/PPE: Use caution, work in a well ventilated area, wear gloves, lab coat, and goggles.