

Fixation with Glutaraldehyde

Materials

	vol (for 500 ml) of stock	mg
1. 137 mM NaCl	34.25 ml	2 M
5 mM KCl	0.83 ml	3 M
1.1 mM Na ₂ HPO ₄	5.5 ml	100 mM
0.4 mM KH ₂ PO ₄	2 ml	100 mM
2 mM MgCl ₂	10 ml	100 mM
2 mM EGTA	10 ml	100 mM
5 mM PIPES	25 ml	100 mM
5.5 mM glucose		495
pH 6.1 (cytoskeleton buffer), warm.		

2. Glutaraldehyde, store in tightly-sealed containers at 4°C.

3. Cytoskeleton buffer with 0.3% (w/v) Triton-X 100, 0.5% glutaraldehyde, 30 ml. Triton X-100 should be pipeted slowly with a p-200 pipetman and a trimmed tip. Wipe the outside surface of the pipet tip before adding to the buffer. Pump up and down several times to rinse out the inside surface. Mix in a fixative bottle, cap tightly, and warm in a water bath.

4. Cytoskeleton buffer with 1% glutaraldehyde, 30 ml. Mix in a fixative bottle, cap tightly, and warm in a water bath.

5. Cytoskeleton buffer with 0.5 mg/ml NaBH₄, 30 ml. Prepare fresh before use in a fixation box. Bubbles should be visible. Store NaBH₄ desiccated in a tightly-sealed container.

6. Phosphate buffered saline, 100 ml, warm.

7. Fixation boxes/dished.

Procedure

1. Rinse coverslip 2x with warm phosphate buffered saline.

2. Fix 1 min in buffer 3. Agitate periodically.
 3. Rinse 2x with cytoskeleton buffer.
 4. Fix 15 min in buffer 4. Agitate periodically.
 5. Rinse 2x with cytoskeleton buffer.
 6. Treat 5 min in NaBH₄. Agitate periodically.
 7. Rinse 2x with cytoskeleton buffer.
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