

Fixation for standard EM

Solutions

0.2 M Sodium cacodylate pH 7.4 - make fresh
8.56 g Na cacodylate/ 200 ml
~2.5ml 0.2N HCl to pH 7.4

0.1M CaCl₂·H₂O

Karnovsky's Fixative 0.5%

4% PFA	12.5 ml 16% PFA
72 mM sodium cacodylate pH 7.4	18.0 ml 0.2M Na cacodylate stock
4 mM CaCl ₂	2.0 ml 0.1M CaCl ₂
0.5% glutaraldehyde	3.125 ml 8% glutaraldehyde
	14.375 ml H ₂ O

Karnovsky's Fixative 2.0%

4% PFA	12.5 ml 16% PFA
72 mM sodium cacodylate pH 7.4	18.0 ml 0.2M Na cacodylate stock
4 mM CaCl ₂	2.0 ml 0.1M CaCl ₂
2.0% glutaraldehyde	12.5 ml 8% glutaraldehyde
	5.0 ml H ₂ O

Procedure

1. Remove medium and gently add 0.5% Karnovsky's fixative. Fix for 1-2 hrs.
2. Remove fix and add 2% Karnovsky's fixative. Fix overnight.
3. Scrape the cells. Centrifuge gently (500 RPM, 5 min). Remove fix.
4. Resuspend pellets in 0.5% Karnovsky's fixative and store at 4°C until ready to send.