

X-Gal Staining

(See also the protocol in “Current Protocols in Molecular Biology”)

1. Wash cells 2 X in Tris / PBS.
2. Fix in 4% paraformaldehyde in PBS; 15 minutes at Room Temp;
5 mL for 10 cm, 2 mL for 6 cm.
3. Wash 3 X in PBS / Tris (2nd wash 10 min).
4. Stain in X-Gal Solution; ~3 mL for 10 cm; stain several hours to overnight at 37C.

Solutions

1 X PBS:

4 % paraformaldehyde: dilute 20 % paraformaldehyde into 1 X PBS

Colloid developer (X-Gal Solution):

X-Gal	1 mg / mL	(Stock: 50 mg / mL in DMF)
K ₄ Fe(CN) ₆	5 mM	(Stock 100 mM)
K ₃ Fe(CN) ₆	5 mM	(Stock 100 mM)
MgCl ₂	1 mM	(Stock 1 M)

In 1 X PBS / Tris

100 mM K_xFe(CN)₆:

K ₄ Fe(CN) ₆	4.2 g	(100 mM)
K ₃ Fe(CN) ₆	3.3 g	(100 mM)

Add 100 mL 1 X PBS / Tris

20 % paraformaldehyde (pfa):

pfa	100 g
hot (60 – 65C) water	300 mL
20 X PBS	25 mL
Bring to 450 mL with DI H ₂ O	
Add NaOH until clears (pH 7-8)	
Cool to 4C	
Filter with 0.45 um filter and aliquot	