

Protocol for LacZ Staining:

List of Reagents:

25% Glut Fix (Calbiochem cat number: 354400)
EGTA (sigma cat number: E4378)
Deoxycholic acid (DOC) (sigma cat number D6750)
NP40 (sigma cat number: 74385)
potassium ferrocyanide (sigma cat number: P3289)
potassium ferricyanide (sigma cat number:P3667)
DMF (Sigma cat number: D4551)
Xgal (Gold biotechnology cat number: X4281C5)

Materials:

Fixative

1X PBS/.2% glutaraldehyde/5mMEGTA pH 8/2mM MgCl₂ (pH 7.3)

500ml:	50 ml 10X PBS	100 ml:	10 ml
	4 ml 25% glut		.8 ml
	5 ml .5 M EGTA pH8		1 ml
	1 ml 1M MgCl ₂		.2 ml

Detergent Rinse:

1X PBS/2mMMgCl₂/.01% DOC/.02% NP-40 (pH 7.3)

100ml:	10 ml 10X PBS
	.2 ml 1M MgCl ₂
	1ml 1 %DOC
	1ml 2%NP40

Staining Solution:

1XPBS/2mMMgCl₂/.01%DOC/.02% NP-40/5mM potassium ferricyanide/5mM potassium ferrocyanide/1mg/ml Xgal/20mM Tris pH 7.3 (pH 7.3)

Xgal: Make 25mg/ml stock in DMF-light sensitive keep in -20C

PROTOCOL:

For cells: Wash cells with PBS 2X 5' each
Fix for 5' and rinse with detergent rinse for 10' 3X
Add staining solution and incubate at 37°C

For embryos: Fix for 15' or 30' at RT
E8-E9 fix for 15'
E10-E12 fix for 30' and rinse with detergent rinse for 30' 3X
Add staining solution and incubate at 37°C

For adult organs: perfuse mouse with same fix for 1 minute. Section on vibratome and post fix 10 min. Then begin washes

If organs will not be sectioned, then cut organs into small sections and fix for 30'

Rinse in detergent rinse for 30' at RT (3X)

Add staining solution/37°C/dark for 2-ON hrs

After staining complete, wash with PBS and fix 2% PFA 2hrs-ON