

**Immunohistochemistry Protocol for Whole-Mount or
Vibratome-Sectioned Tissue PECAM
Antibody Staining**

For use with mouse tissue (whole-mount or vibratome-sectioned), fixed with PFA (2-4%)

1. Block with 0.3% Triton/1X PBS containing 5% Normal Donkey Serum.
 - 2 hours
 - Room temp

2. Incubate with rat anti-mouse CD31 antibody at 1:500 dilution in blocking buffer.
 - 12-15 hours (O/N)
 - 4° C

3. Wash with 1x PBS/0.05% Tween
 - 1 hour
 - Room temp

4. Incubate with donkey anti rat fluorescence conjugated antibody diluted 1:200 in blocking buffer.
 - 3-4 hours
 - 4° C

5. Wash with 1x PBS/0.05% Tween
 - 2 hours-overnight
 - 4° C

Protocol for LacZ Staining (mouse V26)

Stock: .5M EGTA pH 8.0
1M Tris pH 7.3
1M MgCl
2mM MgCl in 1xPBS
1% DOC
2% NP40
Xgal 25mg/ml DMF

Glut Fix

1xPBS/0.2% gluteraldehyde/5mM EGTA pH8/2mM MgCl (pH7.3)

| | |
|---------------------|--------------|
| 500ml: 50 ml 10xPBS | 100ml: 10 ml |
| 4 ml 25% glut | 0.8 ml |
| 5 ml 0.5M EGTA | 1 ml |
| 1 ml 1M MgCl | 0.2 ml |

Detergent Rinse:

1xPBS/2mM MgCl/ 0.01% sodium deoxycholate DOC/ 0.02% NP-40 (pH7.3)

100ml: 10ml 10xPBS
0.2 ml 1M MgCl
1 ml 1% DOC
1 ml 2% NP40

Staining Solution (50ml):

1xPBS/2mM MgCl/0.01% DOC/0.02% NP40/ 5mM Kferricyanate (III)/5mM
Kferrocyanide
(II)/Xgal 1mg/ml /20mM Tris pH 7.3

Add to rinse solution (per 50ml stain)

Kferrocyanide (II): 0.106gm
Kferricyanate (III): 0.0823gm
Tris 1M pH 7.3: 1ml

Add Xgal per amount used :

X-gal 25mg/ml stock in DMF

Protocol:

Fix embryos in Glut fix (E8-9: 10min; E10-12: 20min)
Detergent rinse 3x 30min

Stain :

Add staining solution at 37° C, cover in foil (keep dark) 4hrs-o/n

Wash:

1XPBS- 5min

Fix: 4% PFA 2+hours

4%PFA

20gm PFA (with mask)

450ml ddH₂O

heat H₂O to 50°C

Add 15 drops NaOH from plastic pipet

Soln clears as reaches 60° C

Cool on ice

Add 10ml 10x PBS