LACZ STAINING

1. In tissue culture

- Wash cells twice with PBS.
- Fix the cells 15 min at RT in PEMS + 4% formaldehyde.
  PEMS:
  - 0,1 M PIPES pH 6.9
  - 2 mM $MgSO_4$
  - 1 mM EGTA
- Wash twice with PBS.
- Incubate at 37 °C in humidified incubator with LACZ stain.

  LACZ stain:
  - 50 mM NaPi pH7.3
  - 150 mM NaCl
  - 1.3 mM $MgCl_2$
  - 3 mM $K_4Fe(CN)_6$
  - 3 mM $K_3Fe(CN)_6$
  - 1 mg/ml X-gal (20 mg/ml stock in dimethylformamide, at -20 °C)

NOTE: Reaction is light sensitive, therefore cover the tissue culture plates with aluminium foil.

2. *In situ* (Elles H3)

- Prepare mouse tissue on dry ice.
- Store at -80 °C if necessary.
- Cut cryosections on coated slides.

- Thaw slides ± 5 min.
- Allow fixation for 5 min. on ice in
  0.2% Glutaraldehyde
  0.1 M Phosphate buffer, pH 7.3
  5 mM EGTA
  2 mM $MgCl_2$
- Rinse twice in PBS + 2 mM $MgCl_2$ at R.T. (one rinse = 5 dips).
- 5 min in detergent rinse at R.T.
- Circle section with Dakopen.
- Apply on section 100-200 µl staining solution.

  Staining sol.: - 20 mM Tris, pH 7.3
  - 1 mg/ml X-gal

  - staining sol. should be at R.T. before adding X-gal
  - filtrate through 0.22 µm

- Incubate O.N. at 37 ºC in humidified incubator.

- Rinse twice in PBS + 2 mM $MgCl_2$.
- Rinse once in aqua dest.
- Stain for 2 min in neutral red.
- Rinse three times in aqua dest.
- Rinse once in 70% ethanol.
- Rinse once in 100% ethanol.
- Air dry for 15 min.
- Rinse twice in xylene.
- Leave slides in xylene and mount one by one in DEPEX.

3. Whole mount

- Rinse embryos six times in 0.5 ml PBS.
- Fix embryos for 20 min (longer depending on size) in cold PBS containing:
  - 2% formaldehyde
  - 0.2% glutaraldehyde
  - 0.4% PVP-40
- Rinse embryos again extensively in PBS.
- Transfer embryos to 0.5 ml lacZ stain:

  PBS with:
  - 5 mM $K_4Fe(CN)_6$
  - 5 mM $K_3Fe(CN)_6$
  - 25 mM $MgCl_2$
  - 2 mg/ml X-gal (Bethesda Res. Labs)
- Incubate in the dark for 16 to 24 h at 37 °C in humidified chamber.