

IMMUNOFLUORESCENCE – MEFs

1. Place sterile coverslip into 6-well dish and gelatinize

Cover with sterile 0.2% gelatin in 1X PBS

2. Plate MEFs into coverslips, and culture in complete DMEM medium with 10% FCS. Aim for 60-85% confluence on the day of fixation
3. Aspirate medium and wash once with 1X PBS
4. Aspirate and incubate with 2ml fixative for 10min at room temp
5. Wash twice with 2ml 1X PBS. Store at 4°C covered in PBS or proceed to next step
6. Permeabilize by incubating in 1X PBS/0.5% Triton X-100 for 10 min. at room temp
7. Wash cells 5 times with 1X PBS
8. Block for 1 hour at room temp in 1X PBS/2% FCS
9. Aspirate blocking solution and add 1^o antibody diluted in blocking buffer. Incubate overnight at 4°C
10. Wash 3 times with 1X PBS
11. Add 2^o antibody diluted in blocking solution. Incubate 1 hour at room temp. protected from light
12. Wash 3 times with 1X PBS
13. Invert coverslip onto a slide with a drop of Vectashield with DAPI
14. Seal edges with nail polish and ensure that nail polish is completely dry. Store at 4°C for short term or -20°C for longer term. Good for up to 6 months (or sometimes longer)

Gelatin solution

Dissolve gelatin in 1X PBS for a final concentration of 0.2% (w/v).
Autoclave and store at 4°C

Fixative

Dissolve 1g sucrose in 45ml 1X PBS
Add 4.1 ml formaldehyde.

Make fresh for each use, and discard according to appropriate chemical disposal procedures for formaldehyde

Blocking solution

Add fetal calf serum to 1X PBS for a final concentration of 2% (v/v)