

EdU Proliferation Assay

Materials

- EdU kit baseclick: -20 °C; 2.14; ;chemicals box
- EdU kit baseclick: 4 °C; 2.14; 4F44; kitsBoxes
- PBS
- 4% PFA

Day1

EdU preparation

- thaw DMSO diluted stock (10mM)
- dilute in PBS to 1mM -> working solution

Cells labelling

- add working solution in ratio 1:100 in the wells with media
- incubate 30 min. at 37 °C

Cells fixation

- move to chemical cabinet in genomic room
- aspirate media
- wash with PBS
- fix with 4% PFA for 10 min. at RT
- aspirate
- wash with PBS
- aspirate
- add PBS for storage
- store at 4 °C

Cell labelling EdU kit

- Aspirate PBS
- Permeabilize with 0.1% Triton X-100 in PBS for 20 minutes at RT
- Prepare the EdU staining solution (should be used within 15 min)
- Remove permeabilization solution
- Wash 2 x with 3% BSA in PBS
- Aspirate
- Add EdU staining solution incubate for 30 minutes in dark
- From now on avoid exposure to light
- Remove EdU staining solution
- Wash 3 x with 3% BSA in PBS
- Aspirate
- Add PBS
- Note: You can stop here and store the cells at 4 °C

Staining with primary antibodies

- Aspirate PBS
- Add blocking solution (1% BSA in 0.05% Tween-20 PBS) and incubate for 30 minutes
- Aspirate

- Add antibodies diluted in 1% BSA in 0.05% Tween-20 PBS
- Keep in dark at 4 °C overnight

Day 2

Note: Limit exposure to direct light

Solutions to prepare

- 0.05% Tween 20 in PBS (for 3 washes and antibody incubation)
- Solution of secondary antibody and phalloidine in 0.05% Tween 20 in PBS (together)
- DAPI in PBS (1:5000) (make 10mL stock)
- PBS
- Mowiol

Secondary antibodies incubation

- Aspirate primary antibody.
- Wash with 0.05% Tween 20 in PBS (3x)
- Prepare secondary antibody and phalloidine
- Add secondary antibody to the wells
- Incubate for 1h with secondary antibody and phalloidine
- Aspirate
- Wash 1x with PBS

Dapi counterstaining

- Add DAPI working solution to stain nuclei
- Incubate 5min
- Aspirate
- Wash 3x 5 min with PBS
- Check under microscope
- Mount in Mowiol
- Store at 4 °C in dark

Notes

- Phalloidine staining does not work with EdU staining
- Primary antibodies:
- Secondary antibodies (1:400):
- Phalloidine(1:100):