

Fixing and immunolabelling animal cells (with DNA stain)

The cells are adhered to the top surface of a coverslip in the bottom of the well. Throughout the protocol, keep the cells facing towards you when the coverslip is vertical, or up, when the coverslip is horizontal so you don't lose track of which side they're on.

Materials

2 x coverslips of HeLa cells in culture

6 well plate, Petri dishes, kimwipes, forceps, parafilm, 50 ml beaker

20 ml PBS (Phosphate buffered Saline - Ca²⁺ and Mg²⁺ free)

100ml PBSTA (PBS + Tween and Sodium Azide)

10 ml Karsenti's Solution (PIPES, EGTA, MgSO₄, Triton X)

50 ml cold methanol

50µl monoclonal anti-tubulin antibody, raised in mouse (primary antibody) 1:1000

50µl Alexa488 or 546-conjugated Goat-anti-mouse antibody (secondary antibody) 1:200

50µl 5µg/ml DAPI

Antifade

1. Rinse:

- In your six-well plate, **on each row**, fill the first two wells to the top with PBS. Fill the third well in the plate with Karsenti's solution.
- Using forceps, very carefully transfer a coverslip to each of the first wells.
- Move the coverslips to the second well.
- Move the coverslips to the third well and leave it there for **8 seconds**.
- Dunk the coverslips briefly in the beaker of cold methanol on ice.

2. Fix:

- Fill the first wells of a new plate with cold methanol from the tube in the ice.
- Place the coverslips flat in the cold methanol, and put it in the fridge for 10 minutes.
- Meanwhile, prepare the antibody: Line a petri dish with a circle of parafilm and damp kimwipes around the edges.
- Place two drops of 50µl of the **primary** antibody in the center of the dish.

3. Rehydrate:

- Fill all the wells in the first plate to the very top with PBSTA.
- Remove the six-well plate from the fridge
- Dunk the coverslips 10 times in three wells of PBSTA (one row for each coverslip).

4. Primary antibody:

- Remove the cells from the last well of PBSTA
- Carefully remove excess liquid by blotting the edge of the coverslip on a folded kimwipe on the bench.
- Lower the coverslip **cell side down** onto the droplet of antibody.
- Seal the plate, label it, and place it in the incubator for **one hour**.
- Rinse out all the wells in the six-well plate.
- Refill all the wells with PBSTA. Put the lid on.
- Prepare a second petri dish for **secondary** antibody staining, and put the lid on.

5. Secondary antibody:

- Place two drops of 50 μ l of secondary antibody in the center of the second prepared petri dish.
- Remove the coverslip from the first petri dish and dunk it ten times in each of the three wells containing PBSTA.
- Blot the edge of the coverslip to remove excess liquid and place it *face down* in the secondary antibody.
- Cover and seal the dish, cover it with a piece of foil, and leave it on the benchtop for **one hour**.
- Empty the wells and refill three wells with PBSTA.

6. DNA staining

- Prepare another antibody staining dish and place a drop of 50 μ l of DAPI on the parafilm
- Remove the coverslip from the secondary antibody.
- Dunk the coverslip ten times in each of the three wells of PBSTA.
- Blot excess liquid and place the coverslip *face down* on the drop of DAPI
- Cover the dish with foil
- Leave for **5 minutes** on the bench top
- Empty the wells and refill three wells with PBSTA.

7. Mounting:

- Clean a slide with 70% ethanol and a kimwipe, and label it.
- Place a small droplet of antifade in the center of the slide.
- Remove the coverslip from the DAPI.
- Dunk the coverslip ten times in each of the three wells of PBSTA.
- Blot excess liquid and place the coverslip *face down* on the drop of antifade, taking care not to trap bubbles.
- Carefully blot any excess antifade around the edges of the coverslip by laying a kimwipe over the slide and pressing down gently.
- Seal with nail polish.
- Once the nail polish is dry, store your slide in a slide box in the fridge.