ICC/IF of suspension cells using Cytospin™

Immunocytochemistry/immunofluorescence (ICC/IF) is a technique that uses fluorescent antibodies or dyes to detect target antigens within cells. This protocol describes the procedure for ICC/IF of suspension cells using a Cytospin™ centrifuge.

Materials and reagents

PAP pen
Cytospin™ Centrifuge with Cytofunnels, Cytoslides and Cytoclips (Thermo Scientific)
Phosphate buffered saline (PBS)
Triton X-100
Tween 20
Fixative: methanol or paraformaldehyde
Bovine serum albumin (BSA)
Fluoromount™ mounting media (Sigma)
Coverslips

Preparation

Wash buffer: PBS + 0.01% Tween 20 (PBST)
Blocking buffer: 10% serum and 1% BSA in PBST
Fixative: Use methanol from the freezer, or make 3–4% paraformaldehyde in PBS
Permeabilization: 0.1% Triton X-100 in PBS
Antibody diluent: PBST + 0.1% BSA

Slide preparation

1. Label the frosted end of the Cytoslides using pencil or a xylene-proof pen.
2. Insert a Cytoslide into a Cytoclip, ensuring that the surface with the white circle(s) is uppermost. Slide a double or single Cytofunnel into the clip, with the absorbent surface touching the slide. Gently fasten the clip.
3. Lift the inner container out of the Cytospin and remove the lid. Place a filled Cytoclip in each recess (they should tilt forwards). A maximum of twelve slides can be spun at once and the Cytospin must always be balanced.
4. Pipette the required volume of the cell suspension into the Cytofunnel (or into each side of the Cytofunnel if using a double). Replace the lid of the inner container and secure by pressing down the central button. Replace the inner container into the Cytospin and close the Cytospin lid. Centrifuge for 3 min, using medium acceleration. The cell density, loading volume and spin speed should be optimized for each cell line used.
5. Once the spin is complete, lift the inner container out of the Cytospin and remove the lid. Unload each Cytoclip horizontally to avoid run-off of any residual liquid. Place each slide on a slide tray for ~30 s or until all of the slides are unloaded. Place the used Cytofunnels in a bucket of 1% Virkon and set aside for use in any further spins.
6. Transfer the slides into a slide rack and then plunge into fixative. The incubation time in fixative is usually 10 min for paraformaldehyde or 5 min for methanol. The fixation method should be optimized for each experiment. As soon as fixation is complete, remove the rack from the fixative and plunge into two consecutive containers of PBS to rinse.
7. Slides can be stored in sealed boxes lined with damp paper towels at 4°C.
Blocking, permeabilization and immunostaining

1. Use a PAP pen to draw a circular hydrophobic barrier around each cell spot on the slides and place on a slide tray.

2. Pipette 200 µL of permeabilization buffer onto each cell spot and incubate for 5 min at room temperature.

   Please note, the permeabilization reagent, concentration and length of time will require optimization.

   If the cells were fixed in methanol, no permeabilization step is required, therefore proceed straight to blocking (Step 4).

3. Remove the permeabilization buffer by tapping the slides onto tissue. Transfer the slides into a swing rack and place into a container containing wash buffer. Wash the slides at 100 rpm for 5 min on an orbital shaker, then pour off the wash buffer and replace with fresh. Repeat three times. After the final wash, do not pour off the buffer.

4. Fold some blue roll into a square and cut into narrow strips. Soak the strips in water and then place around the edges of a slide tray.

5. Tap the slides on blue roll to remove the residual wash buffer and re-draw the PAP pen circles if needed. Place the slides on the slide tray, pipette 200 µL of blocking buffer used and incubate for 1 h.

   Please note, the type of blocking buffer used and the incubation time should be optimized.

6. Place the slides into a swing rack and wash 3 times as previously described.

7. Dilute primary antibodies in PBST + 1% BSA. If all primary antibodies are directly conjugated, also include counterstains (for example, DAPI) at this stage. If any of the primaries are unconjugated, include counterstains during the secondary antibody incubation step (Step 11).

8. Tap off any remaining buffer and re-draw the PAP pen circles if needed. Place the slides on the slide tray and pipette 200 µL primary antibody solution onto each cell spot. Incubate overnight at 4°C.

   Please note, primary antibody concentration and incubation time will need to be optimized.

9. Tap the slides onto tissue to remove the primary antibody solution.

10. Place the slides in a swing rack and wash 3 times as before. If all primary antibodies are directly conjugated, move directly onto Step 13.

11. Dilute secondary antibodies in PBST + 1% BSA and include any required counterstains (e.g. DAPI). Re-draw the PAP pen circles if needed. Place the slides on the slide tray and pipette 200 µL secondary antibody solution onto each cell spot. Incubate for 1 h at room temperature.

   Please note, secondary antibody concentration and incubation time will need to be optimized.

12. Tap the slides onto tissue to remove the secondary antibody solution.

13. Place the slides in a swing rack and wash 3 times in PBST as before.

14. Once the washes are complete, add coverslips. Tap off residual wash buffer from the slides onto tissue and lay out on a slide tray. Pipette 30–40 µL of Fluoromount mounting medium onto each spot.

15. Use a pair of clean metal forceps to pick up a coverslip at one corner and gently lower over the slide, allowing the liquid to spread out. Coverslip one slide at a time to avoid drying.

16. Once coverslipping is complete, replace the slide tray lid and store the slides in the fridge until they are imaged. Do not disturb slides until mounting medium is fully dry (~2 h).