Cytokine Immunocytochemistry (ICC) Protocol for Single-Cell Suspensions

Reagents and Materials

Full Name	Short Name	Catalog Number
Microscope slides, either Adhesion (Erie Scientific Co.) or Colorfrost Plus (Fisher)	slides	Erie ER-202B-AD or Fisher 12-550-17
CytoSpin centrifuge (Thermo Fisher) or equivalent (optional, for centrifuge method)		
BD Pharmingen™ ICC fixation buffer	Fixation Buffer	550010
BSA (Sigma)		A43-78
Goat serum (Jackson Immunoresearch Laboratories)		005-000-001
Endogenous Peroxidase Blocking Reagent (DAKO)	Endogenous Peroxidase Blocking Buffer	S2001
Endogenous Biotin/Avidin Blocking Kit (Vector Laboratories)	Endogenous Biotin Blocking Buffer	SP-2001
BD Pharmingen™ antibody diluent for IHC supplemented with saponin (0.1% w/v final concentration) prior to use in procedure	Antibody Dilution Buffer	559148
ICC-compatible antibodies		
PBS		
Hematoxylin (optional)		
Antibody detection kits or reagent		See the following table
Aqua-Mount® mounting medium (Lerner Laboratories)	mounting medium	13800

Suggested Compatible Detection Antibodies

Kit or Set	Contents	Catalog Number
BD Pharmingen™ anti-mouse Ig HRP detection kit	Biotinylated anti-mouse Ig, antibody diluent buffer, streptavidin-HRP, DAB chromogen, DAB buffer	551011
BD Pharmingen™ anti-rat Ig HRP detection kit	Biotinylated anti-rat Ig, antibody diluent buffer, streptavidin-HRP, DAB chromogen, DAB buffer	551013
Goat isotype	Biotin goat-anti-rat Ig, streptavidin-HRP, DAB substrate kit	559286, 550946, 550880

Procedural Notes

- Select antibody detection reagents according to the isotype you want. Several kits are available for your convenience.

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- You can choose whether to immobilize cells by the adherence or centrifugation method.
- Once cells have been washed (see step 4 in the staining procedure), they can be quickly rinsed in tap water, air dried (1 hour, RT), and stored at -80°C for future use.
- Perform all incubation steps except the ICC fixation and DAB steps in a humidified chamber.
- If you use the anti-mouse or anti-rat detection kit, follow the steps in the instruction manual accompanying the kit when instructed to do so in the staining procedure.
- Be sure to mix the working DAB substrate solution immediately before use, and discard any remaining solution immediately after use.

Procedure, cell preparation

1. Harvest cells and wash them twice in PBS using centrifugation (400*g* for 5 min) to remove residual protein.
2. Adjust the cell concentration to 4–5 x 10^6 cells per mL in PBS.
3. Attach cells to slides using either the adhesion *or* centrifugation method.

Adhesion method

1. Clean and label the slides.
2. Wash slides in PBS for 5 minutes at RT.
3. Place slides in a humidified box to prevent them from drying.
4. Place 20 to 50 µL of the cell suspension (at least enough to cover the well) in each well of the adhesion slides and let cells adhere at room temperature (RT) for 20 min.
5. Continue with the staining procedure.

Centrifugation method

1. Assemble the CytoSpin centrifuge's sample chamber, filter card, slide, and racks according to the manufacturer's instructions.
2. Load 100 µL of cells in each sample chamber.
3. Centrifuge the slides at 600 rpm for 2 to 4 min.
4. Remove the slides from the rack and place them on a staining rack.
5. Continue with the staining procedure.

Procedure, staining

1. Fix cells on slides using Fixation Buffer for 15 min at RT.
2. Wash slides three times for 5 min in PBS.
3. Block slides with PBS supplemented with 2% (w/v) BSA for 30 min at RT or 10 min at 37°C.
4. Wash slides twice in PBS and proceed with staining or storage (see Procedural Notes).

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5. When you are ready to stain the cells, incubate the slides with 20 µL of 2% goat serum in Antibody Dilution Buffer for 30 min at RT.
6. Wash slides three times with PBS with 5-min incubations between each wash.
7. Block endogenous peroxidase activity with Endogenous Peroxidase Blocking Buffer (20 µL per well) for 10 min at RT.
8. Wash slides three times in PBS with 5-min incubations between each wash.
9. Block endogenous biotin with the Endogenous Biotin/Avidin Blocking Buffer according to the instructions accompanying the kit.
10. Incubate each well for 1 hour at RT with 20 µL of purified cytokine-specific antibody or appropriate Ig isotype control diluted in Antibody Dilution Buffer. We recommend titrating the antibody (eg, 1.0, 0.5, 0.25, 0.125 µg per test) to determine the optimal concentration for staining.
11. Wash slides three times in PBS with 5-min incubations between each wash.
12. Incubate each well with 20 µL of the biotinylated secondary antibody diluted in Antibody Dilution Buffer for 30 min at RT.
13. Wash slides three times in PBS with 5-min incubations between each wash.
14. Apply 20 µL of streptavidin-HRP to each well on slides and incubate for 30 min at RT.
15. Wash slides three times for 5 min in PBS.
16. Mix the working DAB substrate solution and incubate the cells with it for a maximum of 5 min at RT. Discard the remainder after use.
17. Stop the development of the color reaction by washing with PBS.
18. [Optional] Counterstain with hematoxylin.
19. Mount the slides in short-term storage mounting medium.