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ICC Unfixed Cells—Direct Method

Staining using a fluorophore—conjugated antibody

Research Use Only

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Materials

- Tris buffered saline (TBS) (50 mM Tris, 150 mM NaCl, pH 7.4) or phosphate buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.76 mM KH_2PO_4, pH 7.4)
  (NOTE: for maximum signal intensity, we recommend using TBS with eFluor® Nanocrystals)
- Fixation reagent: 4% formaldehyde in PBS (optional)
- Blocking reagent: IHC/ICC Blocking Buffer-Low Protein (cat. no. 00-4953) or High Protein (cat. no. 00-4952)
- Primary antibody: fluorophore-conjugated format
- Nuclear counterstain: DAPI or DRAQ5 (cat. no. 65-0880)
- Mounting medium: Fluoromount-G (cat. no. 00-4958) or Fluoromount-G with DAPI (cat. no. 00-4959)

Accessories

- Humidified container in which to place the samples during incubations
- Parafilm
- Glass coverslips
- Clear nail polish

Methods

1. Cells are plated at an appropriate density and allowed to attach to the slide or dish (ex. 30,000 cells/chamber in an 8-chamber slide). Cells are usually plated one day prior to staining in order to achieve 60-80% confluency.
2. Cover the cells with blocking solution (100 µL/chamber in an 8-chamber slide). To limit evaporation, use a piece of parafilm to tightly cover the opening of the chamber slide. Incubate in a humidified chamber for 30min at room temperature.
3. Gently wash the cells 3 times in PBS or TBS (5 min/wash). Use a dropper to add PBS or TBS to the chamber followed by aspiration to remove the buffer.
4. Dilute fluorophore-conjugated primary antibody or multiple fluorophore-conjugated antibodies, at manufacturer's recommended dilution, in blocking reagent and overlay onto cells, protecting from light. Tightly cover the opening of the chamber slide with parafilm. Incubate in a humidified chamber for 1-2 hours at room temperature.
5. Remove the parafilm cover and gently wash the cells 3 times in PBS or TBS (5 min/wash) as described in step 3.

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6. Optional: Fixation with 4% formaldehyde diluted in PBS for 15 minutes at room temperature. Wash cells 3 times in PBS or TBS
7. Optional: Nuclei can be counterstained using DAPI or DRAQ5. It is necessary to select a counterstaining agent with a fluorescent emission spectra that does not overlap with the emission spectra of the other fluorophores used in the experiment.
8. Mount and coverslip using Fluoromount-G or Fluoromount-G with DAPI. Seal the edge of the coverglass with clear nail polish.
9. Allow slides to dry for 1-2 hours before visualizing.
10. Slides can be stored at 4°C protected from light if needed.

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