full spectrum cell analysis
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Immunofluorescent staining of intracellular antigens on cultured cells

Research Use Only

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Materials

- Phosphate buffered saline (PBS) (137mM NaCl, 2.7mM KCl, 10mM Na_2HPO_4, 1.76mM KH_2PO_4, pH 7.4)
- Tris buffered saline (TBS) (50mM Tris, 150mM NaCl, pH 7.4)
- Fixation reagent: 4% Formaldehyde in PBS or 100% ice cold MeOH
- Permeabilization reagent: 1% Triton X-100 in PBS (w:v)
- Blocking reagent: 10% normal serum (species from which the secondary antibody is made) , 0.1% Triton X-100 in PBS or TBS
- Primary antibody: Purified or biotinylated format
- Secondary antibody: Biotinylated (for 3-step protocol) or fluorophore-conjugated (for 2-step protocol)
- Visualization reagent: Streptavidin eFluor® 605NC, Streptavidin eFluor® 650NC, or Streptavidin- FITC
- Mounting medium: Fluoromount-G (SouthernBiotech) or Fluoro-Gel (EMS)
- Humidified container in which to place the samples during incubations

Experimental Procedure

1. Cells are plated at an appropriate density and allowed to attach to the slide or dish (ex. 30,000 cells/chamber in a 8-chamber slide). Cells are usually plated one day prior to staining in order to achieve 60-80% confluency.
2. Fix the cells with 4% Formaldehyde for 15 min at room temperature or with 100% MeOH at -20oC for 10 min.
   NOTE: optimal fixation (both reagent and time) depends on the antigen of interest and must be optimized. The times and methods are suggested starting points for optimization.
3. Gently wash the cells 3X in PBS (5 minutes/wash) using a dropper to add PBS to the chamber followed by aspiration to remove the buffer
   NOTE: it is critical from this point on that the cells do not dry out as this will result in increased levels of background staining and difficulty in interpretation of staining results. It is best not to aspirate more than 2 wells at a time in order to reduce the possibility of the cells within the wells drying.
4. Overlay the cells with permeabilization reagent. To limit evaporation, use a piece of Parafilm to tightly cover the opening of the chamber slide and place in a humidified container. Incubate at 37°C for 30 min followed by 10 min at room temperature.
   NOTE: These are suggested starting conditions for optimization.
5. Gently wash the cells 3X in PBS (5 minutes/wash) using a dropper and aspiration as described in step 3.
6. Cover the cells with blocking solution (100ul/chamber in an 8-chamber slide). Incubate in a humidified container for 1 hour at room temperature.

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7. Remove the Parafilm cover and gently wash the cells 3X in PBS (5 minutes/wash) as described in step 3.
8. Dilute primary antibody (at manufacturer's recommended dilution) in blocking reagent and overlay onto cells. Incubate in a humidified chamber for 2 hours at room temperature.
   NOTE: Low abundance antigens may require longer primary antibody incubations times of up to 12 hours at 4°C.
9. Gently wash the tissue 3X in TBS (5 minutes/wash) as described in step 3.
   If using an unconjugated primary antibody, continue to step 10 (3-step protocol).
   If using a biotinylated primary antibody, continue to step 12 (2-step protocol).
10. Dilute the biotinylated or fluorophore-conjugated secondary antibody (at manufacturer's recommended dilution) in blocking reagent and overlay onto the cells. Cover the chamber slide with Parafilm and incubate in a humidified chamber for 1 hour at room temperature.
    NOTE: If using fluorophore-conjugated secondary antibody, protect from light and omit Triton X-100 from blocking reagent.
    NOTE: For maximum signal intensity, we recommend using TBS with eFluor® nanocrystals.
11. Gently wash the cells 3X in PBS or TBS (5 minutes/wash) as described above.
12. Dilute Streptavidin eFluor® 605NC, Streptavidin eFluor® 650NC, or Streptavidin-FITC conjugates (at recommended dilution) in blocking reagent (10% normal serum in TBS, omit Triton X-100) and overlay the cells. Cover the chamber slide with Parafilm and incubate for 30 minutes at room temperature. We recommend starting with a 1:100 dilution of Streptavidin eFluor® 605NC.
13. Gently wash the cells 3X in TBS (5 minutes/wash) as described in step 3.
14. Mount and coverslip using one of the recommended mounting media.
15. If needed, store slides at 4°C protected from light.

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