full spectrum cell analysis
eBioscience

eFluor® Nanocrystals for immunofluorescent staining of frozen tissues sections

Research Use Only

eFluor Nanocrystals for immunofluorescent staining of frozen tissues sections protocol

Materials

- Tris buffered saline (TBS) (50mM Tris, 150mM NaCl, pH 7.4)
- Acetone, Reagent grade
- Blocking reagent: 1% bovine serum albumin in TBS or 10% normal serum from the species from which the secondary antibody was made
- Primary antibody: Purified or biotinylated format
- Secondary antibody: Biotinylated (required only if using purified primary antibody)
- Visualization reagent: Streptavidin eFluor® 605NC (cat# 93-2317) or Streptavidin eFluor® 650NC (cat# 95-2317)
- Mounting medium: Fluoromount-G (SouthernBiotech) or Fluoro-Gel (EMS)
- Humidified container in which to place the samples during incubations

Experimental Procedure

1. Air dry cut sections for 10 minutes.
2. Fix the sections by immersing in acetone for 10 minutes using a coplan jar.
3. Rehydrate the tissue (in a coplan jar) in PBS or TBS for 10 min at room temperature.
   NOTE: it is critical from this point on that the tissue does not dry out as this will result in high levels of background staining and difficulty in interpretation of staining results.
4. Cover the tissue with the blocking reagent for 1 hour at room temperature (100 µL/ tissue section). To limit evaporation of blocking reagent and to help evenly spread the blocking solution over the tissue, use forceps to gently overlay the tissue section with a piece of Parafilm cut to the dimension of the tissue. It is not necessary to stretch the Parafilm or cover the edges of the slide.
   NOTE: PAP Pen ink can interfere with eFluor® signal and is not recommended.
5. Using forceps, gently lift and remove the Parafilm without disturbing the tissue section and immerse the slide with tissue into a coplan jar containing TBS. Using an orbital shaker set to low speed, gently agitate, changing the TBS wash solution 2 more times for a total of 3 washes (5 minutes/wash).
6. Dilute primary antibody (at manufacturer's recommended dilution) in blocking reagent. Overlay the primary antibody solution on the tissue and cover with Parafilm as described in step 4. Incubate in a humidified chamber for 2 hours at room temperature.
   NOTE: Low abundance antigens may require primary antibody incubations times of up to 12 hours at 4°C.
7. Gently wash the tissue 3X in TBS (5 minutes/wash) as described in step 5.
   If using an unconjugated primary antibody, continue to step 8 (3-step protocol).
   If using a biotinylated primary antibody, continue to step 10 (2-step protocol).
8. Dilute the biotinylated secondary antibody (at manufacturer's recommended dilution) in blocking reagent and overlay the tissue. Cover with Parafilm as described in step 4 and incubate at room temperature for 1 hour.
9. Gently wash the tissue 3X in TBS (5 minutes/wash) as described in step 5.
10. Dilute Streptavidin eFluor® 605NC or Streptavidin eFluor® 650NC (at recommended dilution) in blocking reagent and overlay the tissue. Cover with Parafilm and incubate for 30 minutes at room

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temperature, protected from light. We recommend starting with a 1:100 dilution of Streptavidin eFluor® 605NC or Streptavidin eFluor® 650NC.

11. Gently wash the tissue 3X in TBS (5 minutes/wash) as described in step 5.
    NOTE: For maximum signal intensity, we recommend using TBS with eFluor® nanocrystals.
12. Mount and coverslip using one of the recommended mounting media.
13. Slides can be stored at 4°C protected from light.

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