Immunofluorescence Staining

Preparation of slides:

1. Grow cultured cells on sterile glass cover slips at 37°C overnight.
2. Wash sample with PBS twice.
3. Fix cells for 15 minutes with 2 mL of 4% paraformaldehyde solution (pH 7.4 with NaOH in PBS).

Step-by-step procedure:

1. Permeabilize cells by incubating for 15 minutes on ice with 2 mL of 0.1% Triton X-100 in PBS. *Skip this step if IF is applied on cell surface biomarker (ex. CSV)
2. Wash cells 3 times with PBS.
3. Incubate cells for 1 hour with blocking buffer (DAKO S3022).
4. Introduce primary antibodies diluted in blocking buffer (in appropriate dilutions) to the sample.

   (If using mouse MaxPab® primary antibodies, purification will be required, please see below)
5. Incubate the slide in dark humid chamber at 4°C overnight.
6. Wash with PBS for 5 minutes. Repeat 5 times.
7. Incubate cover slips in fluorescein-conjugated secondary antibodies in blocking buffer in a dark humidity chamber at 4°C for 1 hour. *Skip this step if fluorescein-conjugated secondary antibodies is not applied. (Perform all subsequent washes under dim and ambient light source.)
8. Wash sample thoroughly with PBS. Each wash lasting 5 minutes. Repeat 6 times.
9. Mount sample by inverting them onto mounting medium on glass slides.
10. Store slides in dark at 4°C. Observe the slide within 24 hours by fluorescent microscope with suitable filter.

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Purification Protocols for unpurified mouse MaxPab®

Note: Purification step is only required for unpurified MaxPab® product (Catalog#:Hxxxxxxxx-B0x), Hxxxxxxxx-B0xP is purified MaxPab®

1. Wash and equilibrate rProtein A Sepharose with 5 bed volumes of Protein A IgG Binding Buffer (Thermo Scientific), 3 times.
2. Load 50 uL sample containing IgG of interest into the micro-centrifuge tube containing 50 uL rProtein A sepharose, and add 200 uL of binding buffer, total volume 300 uL.
3. The sample and rProtein A sepharose were incubated on a 3D rocking platform at the room temperature for 2hrs.
4. Wash and equilibrate the spin column (Micro Bio-Spin Chromatography Column, Bio-Rad Laboratories) with IgG binding buffer.
5. Add the sample mixture from step 3 to the spin column.
6. Centrifuge at 2000 rpm for 5 seconds to collect flow through.
7. Wash column with 300 uL IgG binding buffer 3 times to remove unbound and non-specifically bound proteins.
8. Elute the bound IgG in 100 uL fractions into micro-centrifuge tubes containing 10 uL 1M Tris, pH 10 respectively. Collect at least 6 fractions.
9. Detect purified protein concentration by OD 280. Collect fractions containing purified protein.
10. Dialyze purified protein against PBS at 4℃.
11. Detect protein concentration by OD 280.
12. Store the purified IgG at -20℃.

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