Immunofluorescence Cell Staining

PROTOCOL

Santa Cruz Biotechnology, Inc.

A. TISSUE CULTURE CELLS

- Grow cultured cells in chambered culture slides overnight at 37° C.
- Wash briefly with PBS and fix cells by one of the following procedures:
  - 5 minutes in -10° C methanol, air dry; or
  - 2 minutes in cold acetone, air dry; or
  - 10–15 minutes in 1-4% formalin in PBS (keep wet).

NOTE: If fixing in formalin, we recommend you include a cell permeabilization treatment. After fixing the cells, wash in three changes of PBS. Then incubate for 3 minutes in a solution of 0.01% Triton X-100 in PBS.

- Wash in three changes of PBS.

B. FROZEN TISSUE SECTIONS

- Freeze tissue block, embedded in cryo-embedding medium, in liquid nitrogen according to standard procedures. Frozen tissue block may be stored at -70° C until ready for sectioning.
- Cut 4–10-micron thick tissue sections using a cryostat.
- Adhere sections to **electrostatically-charged micro slides** (e.g. sc-363562) kept at room temperature.
- Slides may be stored at -70° C. Thaw slides at room temperature prior to fixing and staining.
- Fix slides in cold acetone for 10 minutes and keep refrigerated (or choose other fixation procedure). Wash in three changes of PBS.

C. FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUE SECTIONS

- Fix tissue in formalin and embed in paraffin blocks according to standard procedures.
- Cut 4–6 micron thick tissue sections using a microtome, and apply to **electrostatically-charged micro slides** (e.g. sc-363562).
- Deparaffinize in xylenes using three changes for 5 minutes each.
- Hydrate sections gradually through graded alcohols: wash in 100% ethanol twice for 15 minutes each, then 90% ethanol twice for 15 minutes each.
- Wash in deionized H_2O for 1 minute with stirring.
- Aspirate excess liquid from slides.

NOTE: Alternatively, other non-xylenes clearing agents can be used for tissue deparaffinization (e.g. Histo-Clear).

Optional: Antigen unmasking may be performed at this point. Certain antigenic determinants are masked by formalin fixation and paraffin embedding and may be exposed by one of several methods:

1. Heat treatment (recommended method): Place slides in a container and cover with 10 mM **sodium citrate buffer, pH 6.0** (sc-294091); or with 50 mM **glycine-HCl buffer** (sc-29096), pH 3.5, with 0.01% (w/v) **EDTA** (sc-29092). Heat at 95° C for 5 minutes. Top off with fresh buffer and heat at 95° C for 5 minutes (optimal incubation time may vary for each tissue type). Allow slides to cool in the buffer for approximately 20 minutes. Wash in deionized H_2O three times for 2 minutes each. Aspirate excess liquid from slides.
2. Pepsin: Incubate sections for 10–20 minutes in **0.1% Pepsin** (sc-476554) in 0.01 N HCl at room temperature. Wash slides several times in deionized H_2O. Aspirate excess liquid from slides.
3. Saponin: Incubate sections for 30 minutes in **0.05% Saponin** (sc-280079) in deionized H_2O at room temperature. Wash at least three times in PBS. Aspirate excess liquid from slides.

D. INDIRECT IMMUNOFLUORESCENCE STAINING

For indirect immunofluorescence staining of cells or tissue sections, we recommend the use of either conventional secondary antibodies or SCBT's Mouse IgG Binding Proteins conjugated to fluorescent tags.

- Use suction to remove reagents after each step, but avoid drying of specimens between steps. Use sufficient reagent to cover the specimen (approximately 100–500 µl per slide is adequate).

NOTE: All steps involving fluorophore-conjugated antibodies and fluorophore-conjugated Binding Proteins should be performed in the dark.

- Incubate specimens for 30 minutes with 10% normal blocking serum in PBS or in **UltraCruz® Blocking Reagent** (sc-516214) if a conventional secondary antibody is used. Incubate specimens for 30 minutes in **UltraCruz® Blocking Reagent** (sc-516214) if a Mouse IgG Binding Protein is used.

NOTE: Blocking serum should ideally be derived from the same species in which the secondary antibody was raised.

- Wash with three changes of PBS for 5 minutes each.
- Incubate with primary antibody for 60 minutes at room temperature or overnight at 4° C. Optimal antibody concentration should be determined by titration; recommended range is 0.5-5 µg/ml diluted in PBS with 1.5% normal blocking serum, or diluted in **UltraCruz® Blocking Reagent** (sc-516214).
- Wash with three changes of PBS for 5 minutes each.
- Incubate at room temperature for 60 minutes, in a dark chamber, with either a fluorophore-conjugated secondary antibody diluted in either PBS with 1.5% normal blocking serum, or in **UltraCruz® Blocking Reagent** (sc-516214), or a fluorophore-conjugated Mouse IgG Binding Protein diluted in **UltraCruz® Blocking Reagent** (sc-516214). Recommended concentration range is 1-5 µg/ml. Optimal antibody or binding protein concentration should be determined by titration.
- Wash with three changes of PBS for 5 minutes each.

NOTE: Aspirate as much liquid as possible from the slide without drying the specimen.

- Immediately, mount the coverslip with either an aqueous, or a hard-set mounting medium.

NOTE: For a listing of mounting media for immunohistochemistry and immunofluorescence, including **UltraCruz® Aqueous and Hard-set Mounting Media, with and without DAPI** (sc-24941, sc-516212, sc-359850, sc-516213), please browse the Mounting Media for Staining on our website, www.scbt.com.

- Examine using a fluorescence microscope with appropriate filters. Store slides in a dark location at room temperature, if mounted with Hard-set Mounting Medium, or at 4° C if mounted with Aqueous Mounting Medium.

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E. DIRECT IMMUNOFLUORESCENCE STAINING

For direct immunofluorescence staining of cells or tissue sections, we recommend the use of SCBT's monoclonal antibodies conjugated to fluorescein, phycoerythrin, Alexa Fluor® 488 and Alexa Fluor® 647.

- Use suction to remove reagents after each step, but avoid drying of specimens between steps. Use sufficient reagent to cover the specimen (approximately 100–500 µl per slide is adequate).

NOTE: All steps involving fluorophore-conjugated antibodies should be performed in the dark.

- Incubate specimens for 30 minutes with 10% normal blocking serum in PBS or in **UltraCruz® Blocking Reagent** (sc-516214). If blocking serum is used, it should ideally be derived from the same species in which the primary antibody was raised.
- Wash with three changes of PBS for 5 minutes each.
- Incubate with primary antibody conjugated to a fluorophore for 90 minutes at room temperature, or overnight at 4º C. Optimal antibody concentration should be determined by titration; recommended range is 4–10 µg/ml in either PBS with 1.5% normal blocking serum or in **UltraCruz® Blocking Reagent** (sc-516214).
- Wash with three changes of PBS for 5 minutes each.

NOTE: Aspirate as much liquid as possible from the slide without drying the specimen.

- Immediately, mount the coverslip with either an aqueous, or a hard-set mounting medium.

NOTE: For a listing of mounting media for immunohistochemistry and immunofluorescence, including **UltraCruz® Aqueous and Hard-set Mounting Media, with and without DAPI** (sc-24941, sc-516212, sc-359850, sc-516213), please browse the Mounting Media for Staining on our website, www.scbt.com.

- Examine using a fluorescence microscope with appropriate filters. Store slides in a dark location at room temperature, if mounted with Hard-set Mounting Medium, or at 4° C if mounted with Aqueous Mounting Medium.