Immunofluorescence Protocol for cultured cells

Buffers and reagents

(1) TBS：10mM Tris, 150mM NaCl, pH7.2
(2) TBST: 10mM Tris, 150mM NaCl, 0.1% Tween-20, pH7.2
(3) Fixation buffer：4% Formaldehyde in 1 x TBS, pH7.2
(4) Blocking buffer：5% Normal Goat Serum diluted in TBS
(5) Antibody dilution buffer: 5% Normal Goat Serum diluted in TBS supplemented with 0.3% Triton™ X-100, pH7.2
(6) Secondary antibodies, make choices based on the species source of the primary antibody
a. Primary antibodies are derived from rabbits
ABflo® 594-conjugated Goat anti-Rabbit IgG (H+L) (AS039)

ABflo® 488-conjugated Goat anti-Rabbit IgG (H+L) (AS073)
b. Primary antibodies are derived from mice
ABflo® 594-conjugated Goat anti-Mouse IgG (H+L) (AS054)
ABflo® 488-conjugated Goat anti-Mouse IgG (H+L) (AS076)
(7) DAPI staining solution: 1 μg/mL DAPI (1 mg/mL DAPI diluted in TBS)
(8) Antifade mounting medium
(9) Methanol (optional)

Sample preparation

1. Washing: Remove culture medium from multi-well plate with cells grown on the plate or coverslip. Rinse the cells twice with TBS at room temperature, 5 seconds each time.

2. Fixation: Fix the samples with a freshly prepared 4% neutral formaldehyde solution for 15 minutes at room temperature.

3. Post-fixation washing: Aspirate fixative, rinse 3 times with pre-cooled TBS solution, 5 minutes each time.

4. Neutral formaldehyde permeability: For cytoskeleton, organelles, or nuclear localization proteins, an additional permeability step can be added by treating them with ice methanol on ice for 5-10 minutes (optional).

Note: The fixative of ½ to 1 volume of recommended medium volume of a multi-well plate is sufficient to cover and fix the samples well. Formaldehyde is toxic and should be handled properly, related operations should be done in the fume hood.

Blocking and immunostaining

1.Block cells: Cover cells with 5% Normal Goat Serum. Place coverslips in a humid chamber, while the cell plate could be sealed directly with parafilm. Incubate cells for 30 minutes at 37℃.

2. Primary antibody incubation: Remove the antibody dilution/blocking buffer, drop 2.the primary antibody diluted in TBS gently on the sample until the sample is completely covered, and incubate at 4°C overnight. Place coverslips in a humid chamber, while the cell plate could be sealed directly with parafilm.

Note: The optimal dilution of a primary antibody needs to be characterized based on sample types, target protein abundance, antigen retrieval process, etc.

3. Warming samples: Equilibrate samples to room temperature for 15 minutes, remove the primary antibody staining solution. Wash samples once with TBST for 5 minutes, then repeat 2 more times with TBS, 5 minutes per wash.

4. Secondary antibody incubation: Incubate the samples with proper fluorescent secondary antibodies at 37°C for 1 hour, avoiding light. Wash samples with TBST for 5 minutes, then repeat 2 more times with TBS, 5 minutes per wash.

Note: Fluorescently labeled primary antibodies do not require incubation with secondary antibodies.

5. Nucleus counterstaining: Add 1 μg/mL DAPI staining solution to the samples and incubate for 10 minutes at 37°C, avoiding light. Remove DAPI by washing with TBST for 5 minutes, then repeat 3 more times with TBS, 5 minutes per wash.

6. Mount coverslip with antifade mounting medium. Wipe off excess mounting medium and wait for medium to cure, then seal coverslip with nail polish. The mounted samples can be photographed immediately using fluorescent or confocal microscope. The sample can be stored in the dark at 4°C or -20°C for weeks to months. It is highly recommended that photographing is done within one month of mounting.

Immunofluorescence Protocol for Paraffin Sections

Buffers and reagents

(1) TBS：10mM Tris, 150mM NaCl, pH7.2
(2) TBST: 10mM Tris, 150mM NaCl, 0.1% Tween-20, pH7.2
(3) Antigen retrieval buffers：

0.01M Sodium Citrate buffer (1L)：1.9mM $C_6H_8O_7 \cdot H_2O$,10mM $Na_3C_6H_5O_7 \cdot 2H_2O$, pH 6.0

0.01M Tris-EDTA buffer (1L)：10mM $C_4H_{11}NO_3$,1.0 mM $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$, pH 9.0

(4) Blocking buffer: 5% Normal Goat Serum diluted in TBS
(5)Antibody dilution/blocking buffer:5% Normal Goat Serum diluted in TBS supplemented with 0.3% Triton™ X-100, pH7.2
(6) Secondary antibodies，make choices based on the host species of the primary antibody

a. Primary antibodies are derived from rabbits
Alexa Fluor 594-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) (# AS039)
Alexa Fluor 488-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) (# AS073)
ABflo® 647-conjugated Goat anti-Rabbit IgG (H+L) (#AS060)
Cy3-conjugated Goat anti-Rabbit IgG (H+L) (#AS007)
ABflo® 647-conjugated Goat anti-Mouse IgG (H+L) (#AS059)
Cy3-conjugated Goat anti-Mouse IgG (H+L) (#AS008)

b. Primary antibodies are derived from mice
Alexa Fluor 594-conjugated AffiniPure Goat Anti-Mouse IgG (H+L) (# AS054)
Alexa Fluor 488-conjugated AffiniPure Goat Anti-Mouse IgG (H+L) (# AS076)

(7) DAPI staining solution: 1 μg/mL DAPI (1 mg/mL DAPI diluted in TBS)
(8) Antifade mounting medium
(9) Deionized H_2O
(10) Dewaxing agent
(11) Ethanol
(12) Nail Polish

Dewaxing/Rehydration:

1. Baking slides: Place paraffin sections on a slide rack with the same orientation and heat in a 55° C constant temperature oven for 30 minutes. Simultaneously, heat a container of deparaffinization solution in a 55° C oven.

2. Serial wash: Take out the baked slides rack and incubate it in the following reagents using the Coplin jar:
   (1) Immerse slides in dewaxing agent (toxic-free) 3 times, 5 minutes each time;
   (2) Rehydrate sections by sequentially incubating with absolute ethanol 2 times, 5 minutes each time;
   (3) Rehydrate sections by sequentially incubating with 95% absolute ethanol once and 85% absolute ethanol once, 3 minutes each time;
   (4) Wash slides with deionized H_2O for 5 minutes.

Note: The water flow cannot be directly against the tissue and keeping the tissue moist during washes and rehydration.

Antigen Retrieval (optional) :

Method 1: Microwave heat retrieval

1. Immerse sections in a box containing the antigen retrieval buffer; heat the buffer on a high power setting for 3 minutes, followed by incubation in microwave for 5 minutes.

2. Repeat the heating-incubation cycle once.

3. Continue heating the buffer at a medium-low power setting for 1 minute and let it stand in the microwave for 5 minutes.

4. Take out the box from microwave and cool it to room temperature naturally. Wash sections with TBST 3 times, 1 minute each.

Method 2: High-pressure heat retrieval

1. In a pressure cooker, add pH 6.0 citric acid antigen retrieval solution and heat on high. Once boiling, place the sections into the cooker, ensuring the tissue is completely immersed. .

2. Seal the cooker and continue heating on high until the pressure valve starts venting. Reduce heat to medium and set timer for 3.5 minutes.

3. Remove from heat, rinse under tap water (about 15 seconds), and open the cooker once the temperature of the retrieval solution reaches room temperature.

4. Wash sections with TBST buffer 3 times, 1 minute each.

Note: Ensure sections are fully submerged in the retrieval solution. Do not open the pressure cooker during the retrieval process. Choose the appropriate retrieval solution based on experimental needs. Adjust retrieval time based on tissue condition.

Blocking and immunostaining

1. Blocking: Cover cells or tissue sections with 5% Normal Goat Serum. Cover cells with 5% Normal Goat Serum. Place coverslips in a humid chamber, while the cell plate could be sealed directly with parafilm. Incubate cells for 30 minutes at 37℃.

2. Primary antibody incubation: Remove the antibody dilution/blocking buffer, drop the primary antibody diluted in TBS gently on the sample until the sample is completely covered, and incubate at 4°C overnight. The coverslips should be placed in a humid chamber while the cell plate could be sealed directly with parafilm.

Note: The optimal dilution of a primary antibody needs to be characterized based on sample types, target protein abundance, antigen retrieval process, etc.

3. Warming samples: Equilibrate samples to room temperature for 15 minutes, remove the primary antibody solution. Wash samples once with TBST for 5 minutes, then repeat 2 more times with TBS, 5 minutes per wash.

4. Secondary antibody incubation: Incubate the samples with proper fluorescent secondary antibodies at 37°C for 1 hour, avoiding light. Then, remove the secondary antibody solution and wash samples with TBST for 5 minutes, then repeat 2 more times with TBS, 5 minutes per wash.

Note: Fluorescently labeled primary antibodies do not require incubation with secondary antibodies.

5. Nucleus counterstaining: Add 1 μg/mL DAPI staining solution to the samples and incubate for 30 minutes at room temperature, avoiding light. Remove DAPI by washing with TBST for 5 minutes, then repeat 3 more times with TBS, 5 minutes per wash.

6. Mount coverslip with antifade mounting medium. Wipe off excess mounting medium and wait for medium to cure, then seal coverslip with nail polish. The mounted samples can be photographed immediately using fluorescent or confocal microscope. The sample can be stored in the dark at 4°C or -20°C for weeks to months. It is highly recommended that photographing is done within one month of mounting.

Note: During the experiment, all reagent drops should be accurate, rapid, and sufficient, and the sections should keep humid; The dyeing step starts with the incubation of the second antibody, and attention should be paid to the operation of avoiding light in all subsequent steps. After dyeing, it is necessary to observe and collect images in time to avoid drying and fluorescence quenching.