ICC/IF Protocol

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Abstract

Immunohistochemistry (IHC) is a method that combines biochemical, histological and immunological techniques into a simple but powerful assay for protein detection. IHC provides valuable information as it visualizes the distribution and localization of specific cellular components within cells and in proper tissue context.

This protocol describes the steps for performing ICC/IF.

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Guidelines

Tissue preparation is a key to successful IHC experiments. Since no universal tissue preparation method will be ideal for all sample and tissue types, the protocol given here is intended as a starting point from which the experimenter must optimize as needed. All conditions should be standardized in order to ensure reproducible results. Keep in mind that you must be careful not to allow tissues to dry out at any time.

Protocol

Sample Preparation - Cell Climbing Slices

Step 1.

This fixation procedure using paraformaldehyde and formalin fixatives may cause autofluorescence in the green spectrum. In this case, you may try fluorophores in the (i) red range or (ii) infrared range if you have an infrared detection system.

Sample Preparation - Cell Climbing Slices

Step 2.

Place settled coverslip in culture bottle or perforated plate.

Sample Preparation - Cell Climbing Slices

Step 3.

Take out coverslip after cell growth has reached 60%.

Sample Preparation - Cell Climbing Slices

Step 4.

Wash the coverslip 3X with PBS (AR0030, Boster Bio) to remove culture medium.

REAGENTS

Phosphate Buffered Saline (PBS) Powder AR0030 by Boster Bio

Sample Preparation - Cell Climbing Slices

Step 5.

Immerse the coverslip (cells face up) in cold acetone or 4% paraformaldehyde (AR1068, Boster Bio) or neutral formalin for 10 to 20 minutes. Remember to close the lid to prevent evaporation.

REAGENTS

4% Paraformaldehyde (PFA) Solution in PBS AR1068 by Boster Bio

Sample Preparation - Cell Climbing Slices

Step 6.

Wash the coverslip 3X with PBS.

Sample Preparation - Cell Climbing Slices

Step 7.

Put the coverslip on filter paper (cells face up).

Sample Preparation - Cell Climbing Slices

Step 8.

Remove the liquid on the coverslip and allow it to dry for 8-10 hours.

Sample Preparation - Cell Climbing Slices

Step 9.

To thaw the slice, wash with neutral PBS at room temperature for 10-15 minutes (The cell climbing slice can be stored in gelatin at -20°C for one week).

Inactivation

Step 10.

Mix H_2O_2 with distilled water (v/v: 1:50).

Inactivation

Step 11.

Immerse frozen section or cell climbing slice in the diluted H_2O_2 at room temperature for 10 minutes.

Inactivation

Step 12.

Wash the section 3X distilled water (1 minute each).

Antigen Retrieval (Proteolytic Induced Epitope Retrieval: PIER)

Step 13.

Dry the cell slices with filter paper.

Antigen Retrieval (Proteolytic Induced Epitope Retrieval: PIER)

Step 14.

Add compound digestion solution (AR0022, Boster Bio) (e.g. Trypsin solution or other enzymatic antigen retrieval solution) to the slices. We recommend the addition of 0.1% Triton to the samples before the digestion because this reduces surface tension and allows reagents to easily cover the entire sample.

REAGENTS

Antigen Retrieval Buffer (Enzymatic Digestion) For IHC AR0022 by Boster Bio

Antigen Retrieval (Proteolytic Induced Epitope Retrieval: PIER)

Step 15.

Incubate the slices at room temperature for 10 minutes.

Antigen Retrieval (Proteolytic Induced Epitope Retrieval: PIER)

Step 16.

Wash with 3X PBS (10 minutes each).

Blocking

Step 17.

Add 5% BSA blocking solution or normal goat serum to the PIER-treated samples.

Blocking

Step 18.

Incubate the samples at 37°C for 30 minutes.

TEMPERATURE

37 °C Additional info:

Blocking

Step 19.

Shake off extra liquid and dry the samples with filter paper (No washing required).

Primary Antibody Incubation

Step 20.

Dilute primary antibody with antibody diluent (AR1016, Boster Bio) to the concentration recommended by the antibody manufacturer.

REAGENTS
Antibody Dilution Buffer With BSA, Reducing Background AR1016 by Boster Bio

Primary Antibody Incubation
Step 21.

Add the diluted antibody (Recommended concentration: 0.4 µg to 2 µg) to the samples and incubate at 4°C overnight.

TEMPERATURE
4 °C Additional info:

Primary Antibody Incubation
Step 22.

Wash the samples 3X with PBS (15 minutes each).

Secondary Antibody Incubation
Step 23.

Dilute biotinylated secondary antibody with antibody diluent to the concentration recommended by the antibody manufacturer.

Secondary Antibody Incubation
Step 24.

Add the diluted antibody to the samples and incubate at 37°C for 30 minutes.

TEMPERATURE
37 °C Additional info:

Secondary Antibody Incubation
Step 25.

Wash the samples 3X with PBS (8 minutes each).

Staining
Step 26.

Add Strept-Avidin Biotin Complex – Fluorescence Iso-Thio-Cyanate (SABC-FITC) or Strept-Avidin Biotin Complex – Cyanine-3 (SABC-Cy3) reagents to the samples.

LINK:
https://www.bosterbio.com/catalogsearch/result/?q=SABC+Cy3

Staining
Step 27.

Incubate the samples at 37°C for 30 minutes. Remember to avoid light.

TEMPERATURE

37 °C Additional info:

Staining

Step 28.

Wash the samples 2X with PBS (Total 2 hours).

Staining

Step 29.

Seal the slices with water soluble sealing reagent (AR1018, Boster Bio).

REAGENTS

Aqueous Mounting Medium For IHC, ICC AR1018 by Contributed by users

Staining

Step 30.

Monitor the staining intensity under a fluorescence microscope.

Staining

Step 31.

Counterstain by adding DAPI staining solution (AR1176, AR1177, Boster Bio) to the sample.

REAGENTS

DAPI Solution AR1176 by Boster Bio

DAPI Counterstaining Solution AR1177 by Boster Bio

Staining

Step 32.

Check the staining intensity again under a fluorescence microscope.

Staining

Step 33.

For slide storage without significant decay in fluorescence signal, add 20 µL of anti-fade solution (AR1109, Boster Bio) to the sample followed by a cover glass. Avoid bubbles.

REAGENTS

Antifade Mounting Medium, Prolong Flourescence AR1109 by Boster Bio