IHC-F Protocol Version 2

CJ Xia

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 the immunohistochemistry method with frozen tissue sections.

Citation: CJ Xia IHC-F Protocol. **protocols.io**
dx.doi.org/10.17504/protocols.io.m4hc8t6
Published: 07 Feb 2018

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

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 - Snap Freezing and OCT Embedding

Step 2.

Harvest fresh tissue and place it in a dish filled with ice-cold PBS buffer (AR0030, Boster Bio).

REAGENTS

Phosphate Buffered Saline (PBS) Powder AR0030 by Boster Bio

Sample Preparation - Snap Freezing and OCT Embedding

Step 3.

Wash the tissue thoroughly with PBS to remove blood. Use forceps to remove connective tissues.

Sample Preparation - Snap Freezing and OCT Embedding

Step 4.

Cut the tissue into slices with the thickness of 3 mm or less.

Sample Preparation - Snap Freezing and OCT Embedding

Step 5.

Immediately snap freeze the tissue in iso-pentane cooled in dry ice and keep the tissue at -70°C. Do not allow frozen tissue to thaw before cutting.

TEMPERATURE

-70 °C Additional info:

Sample Preparation - Snap Freezing and OCT Embedding

Step 6.

Prior to cryostat sectioning, position the tissue in a mold (which can be simply made by using tin foil) and cover the tissue completely in Optimal Cutting Temperature (OCT) embedding medium.

Sample Preparation - Snap Freezing and OCT Embedding

Step 7.

Use forceps to take the bottom part of the mold into liquid nitrogen for 1 to 2 minutes. The OCT should change to white.

Sample Preparation - Cryostat Sectioning

Step 8.

Pre-cool a slicer box and detector to -22°C and -24°C respectively. Ensure the completeness and smoothness of the blade.

Sample Preparation - Cryostat Sectioning

Step 9.

Place the tissue from the mold to the detector where the tissue is fixed.

Sample Preparation - Cryostat Sectioning

Step 10.

Quickly and carefully slice the cryostat sections at 5-10 µm and mount them on gelatin-coated histological slides. Note that:

- Use coverslip to take sliced tissue

- Cryostat temperature should be between -15°C and -23°C
- The sections will curl up if the specimen is too cold
- The sections will stick to the knife if the specimen is too warm

Sample Preparation - Cryostat Sectioning

Step 11.

Air dry the sections at room temperature for 30 minutes to prevent them from falling off the slides during antibody incubations.

Sample Preparation - Cryostat Sectioning

Step 12.

Store the slides at -70°C. Note that:

- The slides can be stored unfixed for several months at -70°C
- Frozen tissue samples saved for later analysis should be stored intact

TEMPERATURE

-70 °C Additional info:

Sample Preparation - Cryostat Sectioning

Step 13.

Immediately add 50 µL of ice-cold fixation buffer to each tissue section upon removal from the freezer.

Sample Preparation - Cryostat Sectioning

Step 14.

Fix frozen section by immersing it into 4% paraformaldehyde (AR1068, Boster Bio) at 2-8°C for 8 minutes (or optimally at -20°C for 20 minutes).

REAGENTS

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

Sample Preparation - Cryostat Sectioning

Step 15.

Wash the section 3X with PBS and allow it to dry at room temperature for 30 minutes.

Inactivation

Step 16.

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

Inactivation

Step 17.

Immerse frozen sections or cell climbing slices into the diluted H_2O_2 at room temperature for 10 minutes

Inactivation

Step 18.

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

Antigen Retrieval (Proteolytic Induced Epitope Retrieval: PIER)

Step 19.

Dry the frozen sections with filter paper.

Antigen Retrieval (Proteolytic Induced Epitope Retrieval: PIER)

Step 20.

Add compound digestion solution (AR0022, Boster Bio) (e.g. Trypsin solution or other enzymatic antigen retrieval solution) to the sections or slices.

REAGENTS

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

Antigen Retrieval (Proteolytic Induced Epitope Retrieval: PIER)

Step 21.

Incubate the sections at room temperature for 3 to 5 minutes.

Antigen Retrieval (Proteolytic Induced Epitope Retrieval: PIER)

Step 22.

Wash the sections with 3X PBS (5 minutes each).

Blocking

Step 23.

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

Blocking

Step 24.

Incubate the samples at 37°C for 30 minutes.

TEMPERATURE

37 °C Additional info:

Blocking

Step 25.

Discard extra liquid (No washing required).

Primary Antibody Incubation

Step 26.

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 27.

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

TEMPERATURE
37 °C Additional info:

Primary Antibody Incubation

Step 28.

Wash the samples 2X with PBS (20 minutes each).

Secondary Antibody Incubation

Step 29.

Dilute biotinylated secondary 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

Secondary Antibody Incubation

Step 30.

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

TEMPERATURE
37 °C Additional info:

Secondary Antibody Incubation

Step 31.

Wash the samples 2X with PBS (20 minutes each).

Staining

Step 32.

Add Strept-Avidin Biotin Complex (SABC) HRP-conjugated or AP-conjugated reagents to the samples.

LINK:
https://www.bosterbio.com/catalogsearch/result/?q=sabc+alkaline+phosphatase

Staining

Step 33.

Incubate the samples at 37°C for 30 minutes.

TEMPERATURE

37 °C Additional info:

Staining

Step 34.

Wash the samples 3X with PBS (20 minutes each).

Staining

Step 35.

Add a suitable amount of DAB reagent (AR1025, AR1022, Boster Bio) to the samples and incubate in darkness at room temperature for 10 to 30 minutes.

REAGENTS

DAB Chromogenic Substrate Reagent Kit (Blue) AR1025 by Boster Bio

DAB Chromogenic Substrate Reagent Kit (Yellow) AR1022 by Boster Bio

LINK:

https://www.bosterbio.com/catalogsearch/result/index/?applications=17&q=dab+chromogenic

Staining

Step 36.

Monitor the tissue staining intensity under a bright-field microscope.

NOTES

CJ Xia 05 Jan 2018

If the staining background is too high, wash the section 4X with 0.01-0.02% TWEEN 20 PBS and 2X with pure PBS after the SABC reaction and before DAB staining. Then use DAB to stain the samples.

Staining

Step 37.

Wash the samples 3X to 5X with distilled water

Staining

Step 38.

Counterstain (if necessary)

- Add haematoxylin (AR0005, Boster Bio) to the sample
- Dehydrate
- Immerse the paraffin sections 2X in dimethylbenzene (7 minutes each)

REAGENTS

Hematoxylin Counterstain Solution AR0005 by Boster Bio

Staining

Step 39.

Check the tissue staining intensity under a bright-field microscope.