

IHC-P Protocol

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Abstract

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Protocol

Sample Preparation - Paraformaldehyde Cooling and Dehydration

Step 1.

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 - Paraformaldehyde Cooling and Dehydration

Step 2.

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

Sample Preparation - Paraformaldehyde Cooling and Dehydration

Step 3.

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

Sample Preparation - Paraformaldehyde Cooling and Dehydration

Step 4.

Immerse the slices in 4% paraformaldehyde ([AR1068](#), [Boster Bio](#)) at room temperature for 8 minutes.



REAGENTS

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

Sample Preparation - Paraformaldehyde Cooling and Dehydration

Step 5.

Immerse the slices in 4% paraformaldehyde (pre-cool at 4°C) for 6 to 7 hours. The paraformaldehyde volume should be 20X greater than the tissue volume by weight.

Sample Preparation - Paraformaldehyde Cooling and Dehydration

Step 6.

Wash the tissue 3X with PBS (1 minute each).

Sample Preparation - Paraformaldehyde Cooling and Dehydration

Step 7.

Dehydrate the tissue by immersing the tissue sequentially as follows:

- 1X into 80% ethanol (1 hour at 4°C)
- 1X into 90% ethanol (1 hour at 4°C)
- 3X into 95% ethanol (1 hour each at 4°C)
- 3X into 100% ethanol (1 hour each at 4°C)
- 3X into dimethylbenzene (0.5 hr each at room temperature)

Sample Preparation - Liquid Paraffin Section

Step 8.

Prepare the first portion of liquid paraffin in a suitable bath and allow the paraffin to reach and maintain at 60°C.

 **TEMPERATURE**

60 °C Additional info:

Sample Preparation - Liquid Paraffin Section

Step 9.

Immerse the tissue 2X into the paraffin bath (2 hours each).

Sample Preparation - Liquid Paraffin Section

Step 10.

Prepare the second portion of liquid paraffin in a suitable bath and allow the paraffin to reach and maintain at 60°C.

 **TEMPERATURE**

60 °C Additional info:

Sample Preparation - Liquid Paraffin Section

Step 11.

Pour the second portion of paraffin into a mold.

Sample Preparation - Liquid Paraffin Section

Step 12.

Quickly transport the tissue from the paraffin bath to the mold with paraffin.

Sample Preparation - Liquid Paraffin Section

Step 13.

Incubate the tissue at room temperature until it coagulates.

Sample Preparation - Liquid Paraffin Section

Step 14.

Store the tissue at 4°C.

TEMPERATURE

4 °C Additional info:

Sample Preparation - Section Slicing and Incubation

Step 15.

Secure the paraffin section on the slicer.

Sample Preparation - Section Slicing and Incubation

Step 16.

Slice one to two pieces of the section to adjust the slicer so that the section and blade are parallel

Sample Preparation - Section Slicing and Incubation

Step 17.

Slice the remaining section carefully with 5 µm thickness.

Sample Preparation - Section Slicing and Incubation

Step 18.

Incubate the sliced section in 40 to 50°C water to unfold.

Sample Preparation - Section Slicing and Incubation

Step 19.

Mount the tissue section onto Poly-Lysine ([AR0003, Boster Bio](#)) or APTES ([AR0001, Boster Bio](#)) coated glass slides.

REAGENTS

Poly-L-Lysine Solution, 10X, For 100-200 Slides [AR0003](#) by [Boster Bio](#)

3-Aminopropyltriethoxysilane (APTES) 10ml 50X Concentrated [AR0001](#) by [Boster Bio](#)

Sample Preparation - Section Slicing and Incubation

Step 20.

Incubate the slides overnight at 37°C.

TEMPERATURE

37 °C Additional info:

NOTES

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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.

Dewaxing/Deparaffinization

Step 21.

Prepare the following reagents:

- 90% dimethylbenzene
- 95% dimethylbenzene
- 100% dimethylbenzene
- 90% ethanol
- 95% ethanol
- 100% ethanol

Dewaxing/Deparaffinization

Step 22.

Sequentially immerse paraffin sections into:

- 90% dimethylbenzene (for 7 minutes)
- 95% dimethylbenzene (for 7 minutes)
- 100% dimethylbenzene (for 7 minutes)
- 90% ethanol (for 7 minutes)
- 95% ethanol (for 7 minutes)
- 100% ethanol (for 7 minutes)

Dewaxing/Deparaffinization

Step 23.

Wash the slides with water to remove the ethanol.

NOTES

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The process of dewaxing should be done in a fume hood at room temperature in summer. When the temperature is lower than 18°C, it is recommended to dewax at 50°C.

Inactivation

Step 24.

Immerse dewaxed paraffin section into the 3% H₂O₂ ([AR1108](#), [Boster Bio](#)) at room temperature for 10 minutes.

REAGENTS

3% Hydrogen Peroxide (H₂O₂) Solution, Lab Grade [AR1108](#) by [Boster Bio](#)

Inactivation

Step 25.

Wash the section 3X to 5X with distilled water (total 3 to 5 minutes).

Antigen Retrieval (Heat Induced Epitope Retrieval: HIER)

Step 26.

Immerse the paraffin sections in citrate buffer ([AR0024](#), [Boster Bio](#)).



REAGENTS

Citrate Buffer Powder 2L/Pack, For Heat Antigen Retrieval [AR0024](#) by [Boster Bio](#)

Antigen Retrieval (Heat Induced Epitope Retrieval: HIER)

Step 27.

Heat the buffer in the microwave and turn it off when the buffer has boiled.

Antigen Retrieval (Heat Induced Epitope Retrieval: HIER)

Step 28.

Keep the boiled buffer in the microwave for 5 to 10 minutes.

Antigen Retrieval (Heat Induced Epitope Retrieval: HIER)

Step 29.

Repeat the heating as outlined above for 1 to 2 times.

Antigen Retrieval (Heat Induced Epitope Retrieval: HIER)

Step 30.

Cool the slides until it reaches room temperature.

Antigen Retrieval (Heat Induced Epitope Retrieval: HIER)

Step 31.

Wash the sections 1X to 2X with PBS.

Blocking

Step 32.

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

Blocking

Step 33.

Incubate the samples at 37°C for 30 minutes.



TEMPERATURE

37 °C Additional info:

Blocking

Step 34.

Discard extra liquid (No washing required).

Primary Antibody Incubation

Step 35.

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 36.

Add the diluted antibody to the samples and incubate overnight at 4°C or at 37°C for 1 hour.

Primary Antibody Incubation

Step 37.

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

Secondary Antibody Incubation

Step 38.

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 39.

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



TEMPERATURE

37 °C Additional info:

Secondary Antibody Incubation

Step 40.

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

Staining

Step 41.

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 42.

Incubate the samples at 37°C for 30 minutes.

TEMPERATURE

37 °C Additional info:

Staining

Step 43.

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

Staining

Step 44.

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



REAGENTS

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

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



LINK:

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

Staining

Step 45.

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

NOTES

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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 46.

Wash the samples 3 to 5 times with distilled water.

Staining

Step 47.

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 48.

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