# Immunohistochemistry Protocol for Frozen Sections

**BioLegend, Inc.**

## Abstract

The following is a general procedure guide for preparation and staining of acetone-fixed **frozen** tissues using a purified, unconjugated primary antibody, biotinylated secondary antibody and streptavidin-horseradish peroxidase (Sav-HRP) and DAB detection system. Because each antigen differs in terms of requirement for fixation, amplification step, etc., it is not possible to write an inclusive protocol that will work for all antigens. The user must determine optimal conditions for each antigen of interest. Many protocols for staining individual antigens, as well as useful tips and troubleshooting guides for immunohistochemistry, can be found at the IHC World web site (http://www.ihcworld.com/).

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## Protocol

Prepare frozen tissue sections
**Step 1.**

Place a freshly dissected tissue block (<5 mm thick) on to a pre-labeled tissue base mold.

Prepare frozen tissue sections
**Step 2.**
Cover the entire tissue block with cryo-embedding media (e.g. OCT).

Prepare frozen tissue sections
**Step 3.**
Slowly place the base mold containing the tissue block into liquid nitrogen till the entiretissue block is submerged into liquid nitrogen to ensure tissue is frozen completely.

Prepare frozen tissue sections
**Step 4.**
Store the frozen tissue block at -80°C until ready for sectioning.

Prepare frozen tissue sections
**Step 5.**

Transfer the frozen tissue block to a cryotome cryostat (e.g. -20°C) prior to sectioning and allow the temperature of the frozen tissue block to equilibrate to the temperature of the cryotome cryostat.

Prepare frozen tissue sections
**Step 6.**

Section the frozen tissue block into a desired thickness (typically 5-10 µm) using the cryotome.

Prepare frozen tissue sections

**Step 7.**

Place the tissue sections onto glass slides suitable for immunohistochemistry (e.g. Superfrost).

Prepare frozen tissue sections

**Step 8.**

Dry the tissue sections overnight at room temperature. Sections can be stored in a sealed slide box at -80°C for later use.

DURATION

16:00:00

Immunostain frozen tissue sections

**Step 9.**

Fix the tissue sections with a suitable fixative. One of the commonly used fixation methods for frozen tissue sections is to immerse the slides in pre-cooled acetone (-20°C) for 10 min.

DURATION

00:10:00

Immunostain frozen tissue sections

**Step 10.**

Pour off the fixative and allow acetone to evaporate from the tissue sections for ≥ 20 min at room temperature.

DURATION

00:20:00

Immunostain frozen tissue sections

**Step 11.**

Rinse the slides in 300 ml of 10 mM phosphate buffered saline (PBS) at a neutral pH for 5 min (1/2).

DURATION

00:05:00

NOTES

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A total of 2 changes.

Immunostain frozen tissue sections

**Step 12.**

Rinse the slides in 300 ml of 10 mM phosphate buffered saline (PBS) at a neutral pH for 5 min (2/2).

DURATION

00:05:00

Immunostain frozen tissue sections

**Step 13.**

Incubate the slides in 0.3% $H_2O_2$ solution in PBS at room temperature for 10 min to block endogenous peroxidase activity.

DURATION
00:10:00

Immunostain frozen tissue sections

**Step 14.**

Rinse the slides in 300 ml PBS for 5 min (1/2).

DURATION
00:05:00

Immunostain frozen tissue sections

**Step 15.**

Rinse the slides in 300 ml PBS for 5 min (2/2).

DURATION
00:05:00

Immunostain frozen tissue sections

**Step 16.**

(optional) Add 100 µl blocking buffer (e.g. 10% fetal bovine serum in PBS) onto the sections of the slides and incubate in a humidified chamber at room temperature for 1h.

DURATION
01:00:00

Immunostain frozen tissue sections

**Step 17.**

Drain off the blocking buffer from the slides.

Immunostain frozen tissue sections

**Step 18.**

Apply 100 µl an appropriately diluted primary antibody (in antibody dilution buffer,e.g. 0.5% bovine serum albumin in PBS) to the sections on the slides and incubate in a humidified chamber for 1 h at room temperature or overnight at 4°C.

Immunostain frozen tissue sections

**Step 19.**

Rinse the slides in 300 ml PBS for 5 min (1/2).

DURATION
00:05:00

Immunostain frozen tissue sections

**Step 20.**

Rinse the slides in 300 ml PBS for 5 min (2/2).

DURATION
00:05:00

Immunostain frozen tissue sections

**Step 21.**

Apply 100 µl an appropriately diluted biotinylated secondary antibody (using the antibodydilution buffer) to the sections on the slides and incubate in a humidified chamberat room temperature for 30 min.

DURATION
00:30:00

Immunostain frozen tissue sections

**Step 22.**

Rinse the slides in 300 ml PBS for 5 min (1/2).

DURATION
00:05:00

Immunostain frozen tissue sections

**Step 23.**

Rinse the slides in 300 ml PBS for 5 min (2/2).

DURATION
00:05:00

Immunostain frozen tissue sections

**Step 24.**

Add 100 µl pre-diluted Sav-HRP conjugates (using the antibody dilution buffer) to the sections on the slides and incubate in a humidified chamber at room temperature for 30 min (keep protected from light).

DURATION
00:30:00

Immunostain frozen tissue sections

**Step 25.**

Rinse the slides in 300 ml PBS for 5 min (1/2).

DURATION
00:05:00

Immunostain frozen tissue sections

**Step 26.**

Rinse the slides in 300 ml PBS for 5 min (2/2).

DURATION
00:05:00

Immunostain frozen tissue sections

**Step 27.**

Apply 100 µl DAB substrate solution (freshly made just before use: 0.05% DAB - 0.015%H2O2 in PBS) to the sections on the slides to reveal the color of the antibody staining. Allow the color development for ≤ 5 min until the desired color intensity is reached.

NOTES
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(Caution: DAB is a suspected carcinogen. Handle with care. Wear gloves, lab coat andeye protection.)

Immunostain frozen tissue sections
**Step 28.**

Wash slides in 300 ml PBS for 5 min (1/2).

DURATION
00:05:00

Immunostain frozen tissue sections
**Step 29.**

Wash slides in 300 ml PBS for 5 min (2/2).

DURATION
00:05:00

Immunostain frozen tissue sections
**Step 30.**

(optional) Counterstain slides by immersing sides in Hematoxylin (e.g. Gill's Hematoxylin) for 1-2 min.

DURATION
00:01:00

Immunostain frozen tissue sections
**Step 31.**

Rinse the slides in running tap water for ≥ 15 min.

DURATION
00:15:00

Immunostain frozen tissue sections
**Step 32.**

Dehydrate the tissue slides with  95% alcohol change (1/4).

DURATION
00:05:00

NOTES
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Dehydrate the tissue slides through a total of 4 changes of alcohol (95%, 95%, 100% and 100%), 5 min each.

Immunostain frozen tissue sections

**Step 33.**

Dehydrate the tissue slides with 95% alcohol change (2/4).

DURATION
00:05:00

Immunostain frozen tissue sections

**Step 34.**

Dehydrate the tissue slides with 100% alcohol change (3/4).

DURATION
00:05:00

Immunostain frozen tissue sections

**Step 35.**

Dehydrate the tissue slides with 100% alcohol change (4/4).

DURATION
00:05:00

Immunostain frozen tissue sections

**Step 36.**

Clear the tissue slides in a change of xylene and coverslip using mounting solution (e.g. Permount). [1/3]

NOTES
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Clear the tissue slides in a total of 3 changes of xylene and coverslip using mounting solution (e.g. Permount). The mounted slides can be stored at room temperature permanently.

Immunostain frozen tissue sections

**Step 37.**

Clear the tissue slides in a change of xylene and coverslip using mounting solution (e.g. Permount). [2/3]

Immunostain frozen tissue sections

**Step 38.**

Clear the tissue slides in a change of xylene and coverslip using mounting solution (e.g. Permount). [3/3]

NOTES

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The mounted slides can be stored at room temperature permanently.

Immunostain frozen tissue sections

**Step 39.**

Observe the color of the antibody staining in the tissue sections under microscopy.

## Warnings

**Caution**: DAB is a suspected carcinogen. Handle with care. Wear gloves, lab coat andeye protection.