

# Immunohistochemistry Protocol for Paraffin-Embedded Sections

#### BioLegend, Inc.

#### **Abstract**

The following is a general procedure guide for preparation and staining of formalin-fixed, paraffin-embedded 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 formalin-fixed, paraffin-embedded tissue sections

#### Step 1.

Fix freshly dissected tissue (<3mm thick) with 10% formalin or other fixatives for 24-48 h at room temperature.

**O DURATION** 

24:00:00

NOTES

Kelsey Knight 30 May 2016

(Caution: Formalin is a suspect carcinogen. It can cause eye, skin, and respiratory tract irritation. It should be handled in a hood.)

## Prepare formalin-fixed, paraffin-embedded tissue sections

#### Step 2.

Rinse the tissue with running tap water for 1 h.

**O DURATION** 

01:00:00

Prepare formalin-fixed, paraffin-embedded tissue sections

Step 3.

Dehydrate the tissue through 70% alcohol, 45 min.

**O DURATION** 

00:45:00

Prepare formalin-fixed, paraffin-embedded tissue sections

Step 4.

Dehydrate the tissue through 80% alcohol, 45 min.

**O DURATION** 

00:45:00

Prepare formalin-fixed, paraffin-embedded tissue sections

Step 5.

Dehydrate the tissue through 95% alcohol, 45 min.

**O DURATION** 

00:45:00

Prepare formalin-fixed, paraffin-embedded tissue sections

Step 6.

Dehydrate the tissue through a change of 100% alcohol, 1 h. (1/3)

**O DURATION** 

01:00:00

Prepare formalin-fixed, paraffin-embedded tissue sections

Step 7.

Dehydrate the tissue through a change of 100% alcohol, 1 h. (2/3)

© DURATION

01:00:00

Prepare formalin-fixed, paraffin-embedded tissue sections

Step 8.

Dehydrate the tissue through a change of 100% alcohol, 1 h. (3/3)

**O DURATION** 

01:00:00

Prepare formalin-fixed, paraffin-embedded tissue sections

Step 9.

Clear the tissue through a change of xylene, 1 h. (1/2)

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01:00:00

Prepare formalin-fixed, paraffin-embedded tissue sections

Step 10.

Clear the tissue through a change of xylene, 1 h. (2/2)

**O DURATION** 

01:00:00

## Prepare formalin-fixed, paraffin-embedded tissue sections

#### **Step 11.**

Immerse the tissue in a change of paraffin, 1 h. (1/3)

**O DURATION** 

01:00:00

#### Prepare formalin-fixed, paraffin-embedded tissue sections

#### Step 12.

Immerse the tissue in a change of paraffin, 1 h. (2/3)

© DURATION

01:00:00

#### Prepare formalin-fixed, paraffin-embedded tissue sections

## **Step 13.**

Immerse the tissue in a change of paraffin, 1 h. (3/3)

**O DURATION** 

01:00:00

#### Prepare formalin-fixed, paraffin-embedded tissue sections

#### Step 14.

Embed the tissue in a paraffin block.

#### NOTES

Kelsey Knight 30 May 2016

The paraffin tissue block can be stored at room temperature for years.

## Prepare formalin-fixed, paraffin-embedded tissue sections

#### Step 15.

Section the paraffin-embedded tissue block at 5-8  $\mu m$  thickness on a microtome and float in a 40°C water bath containing distilled water.

#### Prepare formalin-fixed, paraffin-embedded tissue sections

## Step 16.

Transfer the sections onto glass slides suitable for immunohistochemistry (e.g. Superfrost Plus). Allow the slides to dry overnight and store slides at room temperature until ready for use.

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16:00:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

## Step 17.

Deparaffinize slides in a change of xylene, 5 min. (1/2)

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00:05:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

## Step 18.

Deparaffinize slides in a change of xylene, 5 min. (2/2)

**O DURATION** 

00:05:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

## Step 19.

Transfer slides to 100% alcohol, for a 3-minute change. (1/2)

**O DURATION** 

00:03:00

## Immunostain formalin-fixed, paraffin-embedded tissue sections

## Step 20.

Transfer slides to 100% alcohol, for a 3-minute change. (2/2)

**O DURATION** 

00:03:00

# Immunostain formalin-fixed, paraffin-embedded tissue sections

#### **Step 21.**

Transfer through 95% alcohol for 3 min.

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00:03:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

## Step 22.

Transfer through 70% alcohol for 3 min.

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00:03:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

## Step 23.

Transfer through 50% alcohol for 3 min.

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00:03:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### Step 24.

Block endogenous peroxidase activity by incubating sections in  $3\% H_2O_2$  solution in methanol at room temperature for 10 min to block endogenous peroxidase activity.

**O DURATION** 

00:10:00

## Immunostain formalin-fixed, paraffin-embedded tissue sections

#### Step 25.

Change by rinsing in 300 ml of PBS, for 5 min. (1/2)

**O DURATION** 

00:05:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### Step 26.

Change by rinsing in 300 ml of PBS, for 5 min. (2/2)

**O DURATION** 

00:05:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### **Step 27.**

(optional) Perform antigen retrieval to unmask the antigenic epitope. The most commonly used antigen retrieval is a citrate buffer method. Arrange the slides in a staining container. Pour 300 ml of 10 mM citrate buffer, pH 6.0 into the staining container and incubate it at 95-100°C for 10 min (optimal incubation time should be determined by user). Remove the staining container to room temperature and allow the slides to cool for 20 min.

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### **Step 28.**

Change by rinsing slides in 300 ml PBS for 5 min. (1/2)

**O** DURATION

00:05:00

## Immunostain formalin-fixed, paraffin-embedded tissue sections

#### Step 29.

Change by rinsing slides in 300 ml PBS for 5 min. (2/2)

**O** DURATION

00:05:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### Step 30.

(optional) Add 100  $\mu$ l 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.

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**O DURATION** 

01:00:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### **Step 31.**

Drain off the blocking buffer from the slides.

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### Step 32.

Apply 100  $\mu$ l 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 at room temperature for 1 h.

**O DURATION** 

01:00:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### Step 33.

Change by washing the slides in 300 ml PBS for 5 min. (1/2)

**O DURATION** 

00:05:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### **Step 34.**

Change by washing the slides in 300 ml PBS for 5 min. (2/2)

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00:05:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### **Step 35.**

Apply 100  $\mu$ l appropriately diluted biotinylated secondary antibody (using the antibody dilution buffer) to the sections on the slides and incubate in a humidified chamber at room temperature for 30 min.

**O DURATION** 

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## Immunostain formalin-fixed, paraffin-embedded tissue sections

#### **Step 36.**

Change by washing slides in 300 ml PBS for 5 min. (1/2)

**O DURATION** 

00:05:00

## Immunostain formalin-fixed, paraffin-embedded tissue sections

#### **Step 37.**

Change by washing slides in 300 ml PBS for 5 min. (2/2)

#### **O DURATION**

00:05:00

## Immunostain formalin-fixed, paraffin-embedded tissue sections

## Step 38.

Apply 100  $\mu$ l appropriately 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).

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00:30:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### Step 39.

Change by washing slides in 300 ml PBS for 5 min. (1/2)

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## Immunostain formalin-fixed, paraffin-embedded tissue sections

#### Step 40.

Change by washing slides in 300 ml PBS for 5 min. (2/2)

**O DURATION** 

00:05:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### **Step 41.**

Apply 100  $\mu$ l 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 antibody staining. Allow the color development for < 5 min until the desired color intensity is reached.

#### NOTES

Kelsey Knight 30 May 2016

(Caution: DAB is a suspect carcinogen. Handle with care. Wear gloves, lab coat and eye protection.)

## Immunostain formalin-fixed, paraffin-embedded tissue sections

#### Step 42.

Change by washing slides in 300 ml PBS for 2 min. (1/3)

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00:02:00

## Immunostain formalin-fixed, paraffin-embedded tissue sections

#### Step 43.

Change by washing slides in 300 ml PBS for 2 min. (2/3)

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**O DURATION** 

00:02:00

## Immunostain formalin-fixed, paraffin-embedded tissue sections

#### **Step 44.**

Change by washing slides in 300 ml PBS for 2 min. (3/3)

**O DURATION** 

00:02:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### **Step 45.**

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

**O DURATION** 

00:01:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### **Step 46.**

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

**O DURATION** 

00:15:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### **Step 47.**

Dehydrate the tissue slides through a change of 95% alcohol, for 5 min. (1/2)

**O DURATION** 

00:05:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### **Step 48.**

Dehydrate the tissue slides through a change of 95% alcohol, for 5 min. (2/2)

**O DURATION** 

00:05:00

## Immunostain formalin-fixed, paraffin-embedded tissue sections

#### Step 49.

Dehydrate the tissue slides through a change of 100% alcohol, for 5 min. (1/2)

© DURATION

00:05:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### Step 50.

Dehydrate the tissue slides through a change of 100% alcohol, for 5 min. (2/2)

**O DURATION** 

00:05:00

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### Step 51.

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

#### Immunostain formalin-fixed, paraffin-embedded tissue sections

#### **Step 52.**

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

## Immunostain formalin-fixed, paraffin-embedded tissue sections

#### Step 53.

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

#### NOTES

Kelsey Knight 31 May 2016

The mounted slides can be stored at room temperature permanently.

# Immunostain formalin-fixed, paraffin-embedded tissue sections

#### Step 54.

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

## Warnings

Formalin is a suspect carcinogen. It can cause eye, skin, and respiratory tract irritation. It should be handled in a hood.

DAB is a suspect carcinogen. Handle with care. Wear gloves, lab coat and eye protection.