Immunohistochemistry

Yongbo Cui

Abstract

Citation: Yongbo Cui Immunohistochemistry. **protocols.io**
dx.doi.org/10.17504/protocols.io.h8fb9tn
Published: 18 Jun 2017

Protocol

Introduction

Step 1.

This protocol is mainly reference to the protocol on "http://www.immunohistochemistry.us/IHC-protocol.html"

Prepare formalin-fixed, paraffin-embedded tissue sections

Step 2.

1. Formaldehyde fixation: Rinse fresh colon tissue with PBS buffer, then fix tissues with fresh 4% formalin for 24 hours at room temperature. Make sure you have enough fixative to cover tissues. Fixative volume should be 5-10 times of tissue volume.
2. Washing: small flow water rinse formalin fixed colon tissue 3060 minutes, rinse the fixative.
3. Dehydration: Process for paraffin embedding schedule as follow: 70% Ethanol, 1530 minuate; 80% Ethanol, one change, 24 hours; 95% Ethanol, one change, 24 hours; 100% Ethanol (In order to ensure that 100% ethanol anhydrous, ethanol can be placed in the container of anhydrous copper sulfate to absorb moisture), three changes, 1.5 hour each;
4. Transparent: the dehydrated colon tissue is placed in xylene I solution (100% ethanol and pure fresh xylene solution 1:1) for 30 minutes, then placed in xylene II (pure fresh xylene solution) solution for 30 minutes;
5. Soaking wax: Because the selected wax melting point is 5256 ºC, the thermostat is set to 56 ºC, filled with melted wax cup, transparent colon tissue into soft wax cup for 1 hour, then into hard wax dipping wax for another 1 hour;
6. Embedding tissues into paraffin blocks: put the melted hard wax into the embedding metal box, and then quickly colon tissue of hard wax cup by heating the forceps delivery placed in the box, flat bottom, and then embedding frame immediately into the cold water cooling, after about 20 minutes after the solidification of the wax embedding block two blocks; The paraffin tissue block can be stored at room temperature for years.
7. Trim paraffin blocks as necessary and cut at 3-10 um (5 um slice thickness is commonly used, blade angle in 2030 degrees).
8. Show: Place paraffin ribbon in water bath at about 40-45 ºC.
9. Patch: Mount sections onto glass slides, slide the glass on the 60 temperature mounter and place it for 1-2 hours to get a slice.

Deparaffinization and Rehydration

Step 3.

Deparaffinize slides in xylene for 3 times, 5 min each. Then transfer slides to 100% alcohol, for 3 times, 3 min each, and then transfer through 95%, 90% and 75% alcohols respectively for 3 times, 3 min each. At last rinse slides for 5 minutes in running distilled water.

Serum closure

Step 4.

Block endogenous peroxidase activity by incubating sections in 3% H2O2 solution in methanol at room temperature for 10 min to block endogenous peroxidase activity. Rinse with PBS for 3 times, 5 min each.

Antigen retrieval (Optional, recommended)

Step 5.

There are several methods of antigen retrieval. The most common is heat-mediated retrieval in citrate buffer. Arrange the slides in a staining container.

1) Heat slides in 10 mM sodium citrate buffer, pH 6.0 at 95 -100°C for 20 minutes.

2) Remove from heat and let stand at room temperature in buffer for 20 minutes.

3) Rinse in TBST (Tris-Buffered Saline, 0.05% Tween20) 1 minute.

There are other antigen retrieval procedures available and the type of retrieval and the incubation time for antigen retrieval may require some optimization.

Immunostaining

Step 6.

(Do not allow tissues to dry at any time during the staining procedure).

1) Add 100 µl per slide of blocking solution (e.g. 10% fetal bovine serum in PBS) onto the sections of the slides. Incubate 20 to 60 minutes at room temperature.

2) Drain the blocking solution from slides. Apply 100 µl per slide of diluted primary antibody to the sections on the slides at recommended concentration. Incubate 45 minutes at room temperature or overnight at 4°C.

3) Wash slides in 1X TBST 4 times for 5 minutes

4) Apply a 100 µl per slide of diluted conjugated secondary antibody. Incubate for 30 minutes at room temperature.

5) Wash slides in 1X TBST 4 times for 5 minutes.

6) Apply 100 µl 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). Wash slides with PBS for 2 times, 5 min each.

7) Apply color development (i.e. DAB substrate solution, freshly made just before use: 0.05% DAB - 0.015% H2O2 in PBS, follow manufacturer's instructions.) 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. (Caution: DAB is a suspect carcinogen. Handle with care. Wear gloves, lab coat and eye protection.)

8) Wash slides in 1X TBST 4 times for 5 minutes

9) Wash slides in distilled water for 1 minute.

Dehydrate and mount slides:

Step 7.

1). (Optional) Counterstain slides by immersing sides in Hematoxylin for 1-2 min.

2). Rinse the slides in running tap water for 10 min.
3). Dehydrate the tissue slides through 4 times of alcohol (75%, 95%, 100% and 100%), 5 min each.
4). Clear the tissue slides in 3 times of xylene and coverslip using mounting solution. The mounted slides can be stored at room temperature permanently.
5). Observe the color of the antibody staining in the tissue sections under microscopy.