



## Tissue Immunohistochemistry Kit

(Cat. No. PS004)

### Introduction:

Immunohistochemistry is the localization of antigens in tissue sections by the use of labeled antibodies as specific reagents through antigen-antibody interactions that are visualized by a marker such as fluorescent dye, enzyme, radioactive element or colloidal gold.

Albert H. Coons and his colleagues (Coons et al. 1941, 1955; Coons and Kaplan 1950) were the first to label antibodies with a fluorescent dye, and use it to identify antigens in tissue sections. With the expansion and development of immunohistochemistry technique, enzyme labels have been introduced such as peroxidase (Nakane and Pierce 1966; Avrameas and Uriel 1966) and alkaline phosphatase (Mason and Sammons 1978). Colloidal gold (Faulk and Taylor 1971) label has also been discovered and used to identify immunohistochemical reactions at both light and electron microscopy level. Other labels include radioactive elements, and the immunoreaction can be visualized by autoradiography.

Since immunohistochemistry involves specific antigen-antibody reaction, it has apparent advantage over traditionally used special and enzyme staining techniques that identify only a limited number of proteins, enzymes and tissue structures. Therefore, immunohistochemistry has become a crucial technique and widely used in many medical research laboratories as well as clinical diagnostics.

There are numerous immunohistochemistry methods that may be used to localize antigens. The selection of a suitable method should be based on parameters such as the type of specimen under investigation and the degree of sensitivity required. For more information on preparing enzyme-antibody conjugates and the pre-activated enzymes available from [www.gene-rl.com](http://www.gene-rl.com)

### Contents of the kit:

Items	Quantity (100 tests)	Storage conditions	Dilute notice
Perm Buffer (PS0041)	1 bottle (50 ml)	4°C	
10x Wash Buffer (PS0042)	1 bottle (50 ml)	room temperature	*
1x Blocking Buffer (PS0043)	1 bottle (100 ml)	-20°C	
HRP Blocking Reagent (PS0044) (protect from light)	1 vial (500 µl)	4°C	
AEC Peroxidase Substrate Solution (PS0045) (Red color)	1 bottle (30 ml)	4°C	
Hematoxylin (PS0047) (protect from light)	1 bottle (50 ml)	room temperature	
Mounting Medium (PS0048)	1 bottle (30 ml)	4°C	



### Materials/reagents required but not provided:

Primary antibody  
Secondary antibody  
Xylene (for deparaffinization)  
Ethanol (for sample rehydration, etc.)  
Distilled water

### Reagent preparation:

1x Wash Buffer: Dilute 10x Wash Buffer (PS0042) 1/10 with reagent quality water. Store at 4°C.

### Protocol:

#### **Preparation of Slides**

##### **A. For Cell Lines**

1. Grow cultured cells on sterile glass cover slips or slides overnight at 37 °C
2. Wash briefly with PBS
3. Fix as desired. Possible procedures include:
  - 10 minutes with 10% formalin in PBS (keep wet)
  - 5 minutes with ice cold methanol, allow to air dry
  - 5 minutes with ice cold acetone, allow to air dry

4. Wash in PBS

##### **B. For Frozen Tissues**

1. Fix tissue samples with Tissue Fix Kit (PS001).
2. Place the tissue in the cryomold, overlay with Tissue-Tech OCT Compound (PS002), freeze quickly on liquid N<sub>2</sub>. Store frozen blocks at - 80 °C.
3. Cut 4-8 um thick cryostat sections and mount on superfrost plus slides or gelatin coated slides. Store slides at - 80 °C until needed.
4. Dry slides with tissue sections for few minutes at 37°C.
5. Fix in ice cold acetone for 5 minutes. Air dry for 30 minutes.
6. Wash in PBS.

##### **C. For Paraffin Sections**

1. Cut sections and place on slides.
2. Melting paraffin wax in 60°C oven.
3. Place at room temperature let the wax solidified and sections fixed on slides firmly.
4. Transfer the sections in xylene for 3 min.
5. Dry sections for few minutes at 37°C.
6. Fix the sections in 95% ethanol for 30 seconds at 4°C, air dry.
7. Transfer to 70% ethanol for 3 minutes.
8. Transfer to 40% ethanol for 3 minutes.



9. Transfer to distilled water for 3 minutes, air dry.
10. Follow procedure for pretreatment as required.

### **Pretreatments of Tissue Sections**

Antigenic determinants masked by formalin-fixation and paraffin-embedding often may be exposed by epitope unmasking, enzymatic digestion or saponin, etc. **Do not use this pretreatment with frozen sections or cultured cells that are not paraffin-embedded.** The different treatments are summarized below:

Epitope Retrieval: Heat paraffin sections in steamer/water bath/microwave for 20-40 minutes in one of the epitope retrieval solutions and allow sections to cool for 20 minutes.

Enzyme Digestion: Incubate sections at 37 °C for 10-30 minutes in different enzyme solutions such as proteinase K, trypsin, pronase, pepsin, etc. Allow sections to cool for 20 minutes.

### **Immunoenzyme Staining**

1. Thaw slides at room temperature for 10 minutes.
2. Re-hydrate the slides with Perm Buffer (PS0041) for 15 minutes.
3. Wash slides for three times with 1x Wash Buffer.
4. Incubate sample with 1-3 drops Blocking Reagent (10 ml 1x Blocking Buffer (PS0043) + 25  $\mu$ l HRP Blocking Reagent (PS0044) mix well) for 40 minutes. Drain slides and wipe excess Blocking Reagent before proceeding to the next step. Do not rinse.
5. Rinse sample with 1x Wash Buffer, drain slides and wipe excess solution.
6. Incubate sample with primary antibodies. Follow manufacturer recommendations regarding working dilution, time and temperature of incubation. Dilute primary antibodies with 1x Blocking Buffer (PS0043) (usually 50x to 100x dilution).
7. Incubate samples with 1-3 drops of diluted primary antibodies for 1 hour at room temperature
8. Wash sample three times in 1x Wash Buffer (PS0042) for 5 minutes each and drain slides.
9. Dilute secondary antibody with 1x Blocking Buffer (PS0043).
10. Incubate sample with 1-3 drops of secondary antibodies for 30-60 minutes. Adjust the incubation time depending on the thickness of the section. (usually 30 minutes at room temperature)
11. Wash sample three times in 1x Wash Buffer for 5 minutes each and drain slides.
12. Incubate sample with 1-2 drops of Substrate Solution (1  $\mu$ l HRP Blocking Reagent (PS0044) + 1 ml AEC Peroxidase Substrate Solution (PS0045) ) for 30 minutes.
13. Wash sample three times in 1x Wash Buffer for 2 minutes each.
14. Rinse in distilled water and discard additional water.

### **Counterstain**

1. Counterstain with Hematoxylin (PS0047) if desired
2. Add 1-2 drops of Hematoxylin (PS0047) to cover entire tissue for 1-5 minutes. Monitor intensity of tissue staining under a microscope.
3. Wash sample three times in 1x Wash Buffer.
4. Rinse in distilled water and drain slides.



4. Add 1 drop of water to cover entire tissue.
5. Add 1-2 drops of mounting medium (PS0048) for mounting.
6. Place slides vertically on a filter paper or towel to drain excess mounting medium and let dry. Slides are ready for observation under the microscope.

### Troubleshooting:

#### **Lack of Staining**

1. **Lack of antigen:** Check protein expression by *in situ* hybridization. (in some rare cases translation may be blocked even though mRNA is detected)
2. **Antibodies do not work due to improper storage:** Aliquot antibodies into smaller volumes sufficient to make a working solution for a single experiment. Store aliquots in a freezer (-20 to -70°C) and avoid repeated freeze-thaw cycles.
3. **Inactive primary antibodies:** Replace with a new batch of antibodies.
4. **Inadequate tissue fixation:** Try different fixatives.
5. **Tissue overfixation:** Reduce duration of fixation or fix tissues at 4°C.
6. **Incompatible secondary and primary antibodies:** Use secondary antibody that will interact with the primary antibody. For example, if primary antibodies were raised in rabbits, use anti-rabbit secondary but not anti-mouse or anti-goat.
7. **Antigen was destroyed before incubation with primary antibody:** Quenching of endogenous peroxidase was done prior to the addition of primary antibodies. Block peroxidase after incubation with primary antibody.

#### **Overstaining**

1. **High concentration of primary and/or secondary antibodies and/or reagent:** Determine optimal concentration for each component of immunohistochemical reaction: primary antibodies; secondary antibodies; enzymes catalyzing formation of color precipitates.
2. **Long incubation time:** Determine optimal incubation time for each component of immunohistochemical reaction: primary antibodies; secondary antibodies; enzymes; chromogenic substrates.
3. **Non-specific binding of primary and/or secondary reagents to tissues:** Treat tissues to reduce or block non-specific binding of immunohistochemical components to tissues.

#### **High Background**

1. **Tissues have endogenous molecules which are also used in incubation mixtures, for example, the presence of peroxidase in blood cells or the presence of autofluorescent pigment lipofuscin in neuronal cells:** Incubate with normal serum obtained from species other than from the species that were the source of the primary antibody; Block endogenous component. Endogenous peroxidase may be blocked by incubating tissues for 30 minutes at room temperature with 0.3% H<sub>2</sub>O<sub>2</sub> in methanol, or switch to protocols utilizing substances which are not present in tissues.



**Usage:**

Not for human use. For research only. Not for Diagnostic or therapeutic use.