

# User's Manual and Instructions

## ImmuChem Immunohistochemistry (IHC) Kit

**Catalog Number: K3181100 and K3181500**

### Introduction

Biochain provides a reliable and convenient immunohistochemistry detection kit to identify specific gene expression on tissues or immobilized cells. Each kit contains all reagents, including buffer and chromogen, required for the immunohistochemistry. A biotinylated, and affinity purified secondary **anti-mouse IgG** antibody is used to detect primary antibody-antigen complexes adhered to a glass microscope slide, followed by reaction with an enhanced detection reagent. In order to prevent DAB pollution, this kit has a special reagent to make DAB substrate non-toxic and easy to dispose.

### Features

- Simple to use - A highly optimized procedure guides you through each step of the process of the IHC and the counterstaining.
- Maximum sensitivity - Strong signal with low back ground
- Environmental protection - Carcinogenic DAB substrate can be neutralized before disposal

### Application

- This kit can be used for both frozen and paraffin embedded tissue sections

### Description

BioChain's ImmuChem kit contains all reagents, including normal serum, biotinylated anti-rabbit IgG, Solution A, Solution B, liquid DAB, DAB buffer, and detoxification buffer, for a whole procedure of immunohistochemistry. A simple protocol and recommended experimental conditions are provided. Each kit is enough for immunostaining 100 (Cat.#; K3181100 ) or 500(Cat.#; K3181500 ) standard microscope slides and is stable for 1 year at 4°C

### Quality Control

In order to guarantee the kits' quality, each lot of the ImmuChem kit is tested by a standard immunohisto-chemistry protocol with Mouse anti-p53 monoclonal antibody to show the positive staining.

### Contents

Item	K3181100 Amount	K3181100 Part No.	K3181500 Amount	K3181500 Part No.
Normal serum	250 µl	K3181100-1	1.25 ml	K3181500-1
Biotinylated anti-mouse IgG	250 µl	K3181100-II-2	1.25 ml	K3181500-2
Solution A	260 µl	K3181100-3	1.3 ml	K3181500-3
Solution B	260 µl	K3181100-4	1.3 ml	K3181500-4
1x DAB buffer	10.0 ml	K3181100-5	50.0 ml	K3181500-5
Liquid DAB	250 µl	K3181100-6	1.25 ml	K3181500-6
Detoxification	1.0 ml	K3181100-7	5.0 ml	K3181500-7

**Note: This kit is compatible with mouse IgG primary antibodies. If customers' primary antibodies are not mouse IgG, please order Biotinylated antibody separately.**

## Protocol

### DAY 1:

#### 1. Preparation of slides:

- 1) Deparaffinization: soak tissue slides with xylene twice, each time for 15 minutes
- 2) Rehydration: incubate slides in the following graded series of ethanol: 100%I, 100%II, 95%, 90%, 80%, 70%. 5 minutes for each solution. Then incubate in the water for 5 minutes.

**Note: The step 1 is only for paraffin embedded slide. For frozen slide, let the frozen slide dry in room temperature after take it out from the freezer, soak the slide in water for 5 minutes, and go to step 2.**

#### 2. Protocol for Immunostaining:

- 1) Immerse the slides in 0.3% H<sub>2</sub>O<sub>2</sub> (in distilled water) for 30 minutes at room temperature. **For frozen sections, the incubation time should be 10-30 minutes, and for frozen tissue arrays, the incubation time should be 5-10 minutes. Frozen sections might drop off the glass if the incubation time is too long**
- 2) Rinse the slides with water followed by 1x PBS (pH7.4) once, circle the tissue section with a Pap Pen (or Para-Pen, ImmEdge Pen)
- 3) Incubate the slide with 1% Normal serum/PBS [Mix 3.5 ml 1x PBS, pH 7.4 with 1 drops (about 35 µl/drop) of normal serum in a tube] for 30 minutes at room temperature
- 4) Drop off the normal serum from the slides
- 5) Incubate the sections with the PBS diluted first antibody (optimize the antibody titer before starting IHC) in a humid chamber for 1 hour to overnight at room temperature.

### DAY 2:

- 6) Rinse the slides with 1xPBS for 3 times, each time for 5 minutes
- 7) Incubate the slides with PBS diluted Biotin-labeled secondary antibody for 30 minutes at room temperature. [Mix 1.4 ml 1xPBS pH 7.4 and 1 drop (about 35 µl/drop) of **biotinylated anti-mouse IgG** in a tube]
- 8) Rinse the slides with 1xPBS for 3 times, each time for 5 minutes
- 9) Preparation of detection solution: Mix 1.33 ml 1xPBS, 1 drops (about 35 µl/drop) of **Solution A**, and 1 drops (about 35 µl/drop) of **Solution B** in a tube. Incubate the mixture at room temperature for 30 minutes before use.
- 10) Add the detection solution prepared at step 9) to the tissue section. Incubate room temperature for 30 minutes.
- 11) Rinse the slides with 1xPBS for 3 times, each time for 5 minutes.
- 12) Make fresh Development solution: Mix 1.6 ml **DAB buffer**, and 1 drop (about 35 µl/drop) of **Liquid DAB** in a tube.
- 13) Add the Development solution to cover the tissues, and develop for 5-30 minutes
- 14) Stop the reaction by soak the tissue in water.
- 15) Counter stain the slides if necessary (Use Harris' Hematoxylin to stain the nuclear, and use eosin Y to stain cytosol)
- 16) Dehydrate by soaked in the graded series of alcohol: 70%-80%-90%-95%-100%I-100%II, 3 minutes for each solution; then incubated in Xylene twice, 10 minutes each.
- 17) Mounting the slides (Fisher's permount Cat. # SP15-100 or similar mounting material can be used)

Add 2 drops (about 35 µl/drop) of detoxification solution into the used development solution. Incubate for 1 hour or overnight. Then it can be discarded.