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IHC Prep & Detect Kit for Mouse Primary Antibody User Manual

Cat.NO.: PK10018

Description:

The PK10018 kit is designed for developing a complete IHC workflow using primary antibodies raised in mice. The kit contains all the basic reagents, starting from antigen retrieval buffer to mounting media, that would be required for IHC staining of tissue sections with mouse primary antibodies. By simply optimizing the primary antibody concentration and a few operational parameters, a complete IHC workflow can be easily established using this kit.

What's included:

Part NO.	Component	Size
HC001	Antigen Retrieval Buffer (50× Tris-EDTA)	100mL
HC002	Washing Buffer (30×)	120mL
HC003	Blocking Buffer	5mL
HC000	Quenching Buffer (RTU)	5mL
HC004	Primary Antibody Diluent (RTU)	100mL
HC005M	Polymer HRP-Goat anti-Mouse Secondary Antibody	5mL
HC006	Chromogen Component A	0.2mL
HC007	Chromogen Component B	4mL
HC008	Signal Enhancer	5mL
HC009	Counter Staining Reagent	5mL
HC0010	Mounting Media	5mL

What's not included:

Mouse Sourced Primary Antibody

Xylene

Ethanol

Pure water (ddH_2O)

Coplin Jars or Beakers

Slide Basket

Foil or Material to Cover Beaker

Heating Element

Slide Basket Container

Hydrophobic IHC Pen (Optional)

Thermometer

Wash Bottle

Wet Box

Microcentrifuge Tubes

Pipette and Tips

Cover Slides

Warning:

Use proper protective equipment when working with xylene. Xylene can affect you when inhaled or upon contact with skin.

• Contact can irritate skin and eyes.

• Inhaling can irritate the nose and throat and cause coughing or wheezing.

• Exposure can cause headache, dizziness, lightheadedness, and passing out. Repeated exposure can affect concentration, memory, vision, and muscle coordination.

• Use proper personal protective equipment including gloves, protective clothing, eye protection, and adequate ventilation when handling xylene.

• Dispose off xylene and all kit waste properly.

Tips for using this kit:

To maximize the use of the reagents in this kit, we recommend:

When using the dropper bottles, apply the recommended number of drops onto the slide and use the tip of the dropper bottle to distribute the liquid evenly across the tissue section. If the tissue is not completely covered, add 1-2 additional drops of reagent and repeat.

If a hydrophobic pen is not being used, keep slides as flat as possible to prevent the liquid from running off.

Step 0: Paraffin Removal & Rehydration

1. Prepare slides from tissues sections following routine methods. Label slides with ink that is insoluble in xylene and ethanol and place slides in basket or coplin jar.

    * Use an ink insoluble in xylene and ethanol such as a graphite pencil.

2. Immerse slides in xylene for 20 minutes. Repeat once using a separate container with fresh xylene.
3. Immerse slides in a 100% ethanol tank for 5 minutes. Repeat once in fresh 100% ethanol.
4. Immerse slides sequentially in each of the following ethanol solutions for 5 minutes each:
    - 95% ethanol
    - 80% ethanol
    - 60% ethanol
5. Rinse twice in fresh ddH_2O for 1 minute each time.

Step 1: Antigen Retrieval

This kit contains an antigen retrieval buffer based on Tris-EDTA by default, which in our experience is suitable for over 90% of targets. However, if a negative result is obtained using this buffer, we recommend trying alternative buffers listed below.

 # PR30001 Sodium Citrate Antigen Retrieval Buffer 50x

 # PR30014 Protease K Antigen Retrieval Buffer (Heating is not required with this buffer)

1. Use concentrated 50x Antigen Retrieval Buffer (Part NO. HC001) to prepare a 1x solution by adding the concentrated buffer to ddH_2O. For example, to make 500mL of 1x working solution, dilute 10mL HC001 with 490mL ddH_2O.

    * 500mL of prepared 1x antigen retrieval buffer in a 1L beaker should be suitable for 1 or 2 baskets. Calculate how much of each buffer will be needed for the experiment to prepare an appropriate amount of working solution. The working solution has a shorter shelf life than the concentrate (same hereinafter).

2. Heat 1x buffer solution to 95-98˚C with a heating element.

    * Cover the beaker with a lid or foil to avoid vaporizing/vapor inhalation.

3. Place slide basket into heated antigen retrieval buffer. Maintain this temperature while incubating for 15-20 minutes.
4. Remove the beaker from heat and let it cool to room temperature (takes 35–40 minutes).

Step 2: Quenching and Blocking

Based on our experience, endogenous peroxidase quenching is not necessary in most cases and can sometimes even have adverse effects on staining. However, we have included a quenching reagent in this kit since the quenching step is widely recommended in publications.

1. Use 30x Washing Buffer (Part NO. HC002) to prepare a 1x solution by adding concentrated washing buffer to ddH_2O.

To make X mL of 1x working solution, dilute X/30 mL of HC002 with X-X/30 mL or 29X/30 mL of ddH_2O. For example, to make 600mL of 1x working solution, dilute 20mL HC002 with 580mL ddH_2O.

2. Wash slides by rinsing with ddH_2O and then briefly immersing in 100 mL of 1x washing buffer.
3. Drain off the liquid on the slides and absorb any residual buffer around the tissue section (make the slides as dry as possible). Use a hydrophobic IHC pen to draw a circle on the glass slides around tissue sections (optional).
4. Quenching (Optional): Use the ready-to-use Quenching Buffer (Part NO. HC000) dropper bottle to add 2-4 drops onto the slide. Leave the slides at room temperature for 10 minutes. Briefly wash the slide with 1x washing buffer after quenching.
5. Blocking: Use the Blocking Buffer (Part NO. HC003) dropper bottle to add 2- 4 drops directly on slides, covering the whole tissue and block slides at room temperature for 30 minutes inside a wet box.

* Make sure there are no air bubbles in the blocking buffer on the slide.

* TIP: Use the tip of the dropper bottle to distribute the liquid evenly covering the entire tissue section.

* Do NOT let the slides dry out.

6. Drain blocking buffer off the slide and absorb any residual buffer around the tissue section after blocking.

Step 3: Primary Antibody

1. Use the Primary Antibody Diluent (Part NO. HC004) in the kit to dilute your primary antibody (ONLY primary antibodies raised in mice should be used with this kit). It is highly recommended to test several dilutions of the primary antibody, with a starting concentration of 1~10 μg/ml. Apply 80-200 μL (depending on the size of the tissue) of the diluted primary antibody to the slide, covering the entire tissue.

* Calculate the volume of the working solution of the primary antibody and use the antibody diluent to dilute the primary antibody. Preparing too much of the primary antibody working solution is not recommended until an optimal dilution factor has been obtained. The diluted working solution is stable for at least 3 months when stored at 4 °C.

2. Incubate at room temperature in a wet box with a lid for 1 hour.

* Do NOT let the slides dry out.

3. Prepare a 1x washing buffer solution using the 30x Washing Buffer (Part No. HC002) and ddH_2O. Add the prepared buffer to a wash bottle and coplin jar or beaker.

4. Use wash bottle to rinse primary antibody off slides. Then immerse slides in 1x washing buffer for 1 minute. Repeat the rinse and wash steps one more time; drain excess buffer and absorb any residual buffer around the tissue section.

* If different primary antibodies are being used in the same experiment, wash in separate containers of washing buffer.

* Use fresh washing buffer for each wash.

Step 4: Secondary Antibody

1. Use the ready-to-use Polymer HRP-Goat anti-Mouse Secondary Antibody (Part NO. HC005M) dropper bottle to add 2-4 drops directly on the slide, covering the entire tissue.

* The secondary antibody provided in this kit will work ONLY when a primary antibody raised in mouse is used in the previous step.

* TIP: Use the tip of the dropper bottle to distribute the liquid evenly covering the entire tissue section.

2. Incubate at room temperature for 30 minutes inside a wet box.

* Do NOT let the slides dry out.

3. Immerse slides in washing buffer for 1 minute. Repeat two more times using fresh washing buffer each time.

4. Drain the liquid off the slides and absorb any residual buffer around the tissue section.

Step 5: Signal Development

* Take out hematoxylin and bring to room temperature ahead of the counter staining step.

1. Use pipette to prepare an appropriate volume of Chromogen in a microcentrifuge tube. Ratio of solutions Chromogen component A (Part NO. HC006) to Chromogen component B (Part NO. HC007) is 1:20.

* Depending on the size of the tissue sample, approximately 30-80 µl/slide is needed.

2. Use pipette to add enough Chromogen to cover the tissue. Lay slides flat on the benchtop at room temperature for 5 minutes, or until a brown color develops. If color is visible, stop reaction early by washing with 1x washing buffer.

3. Use wash bottle to rinse slides with 1x washing buffer. Then immerse slides in ddH_2O 3-4 times for 30 seconds each time using fresh ddH_2O for each wash. Drain excess buffer off slides and absorb any residual buffer around the tissue section.

Step 6: Signal Enhancement

1. Use the Signal Enhancer (Part NO. HC008) dropper bottle to add 2-4 drops directly onto the slides, covering the entire tissue. Incubate for 5 minutes at room temperature.

* TIP: Use the tip of the dropper bottle to distribute the liquid evenly covering the entire tissue section.

2. Immerse slides briefly in 1x washing buffer to rinse. Drain excess buffer off slides.

Step 7: Counterstain

1. Use the ready-to-use Counter Staining Reagent (Part NO. HC009) dropper bottle to add 2-4 drops directly on the slide, covering entire tissue. Incubate at room temperature for 2-3 minutes.

* TIP: Use the tip of the dropper bottle to distribute the liquid evenly covering the entire tissue section.

2. Immerse slides in washing buffer for 1 minute and then immerse in ddH_2O for 1 minute. Repeat the ddH_2O wash step two more times using fresh ddH_2O for each wash.

Step 8: Mounting

1. Immerse slides sequentially in each of the following ethanol solutions for 5 minutes each:

- 60% ethanol
- 80% ethanol
- 95% ethanol
- 100% ethanol

2. Immerse slides twice in xylene for 5 minute each time using fresh xylene. Drain excess liquid off slides and place slides in a safely ventilated area (such as a fume hood) to allow the residual liquid to evaporate.

3. Use the Mounting Media (Part NO. HC0010) dropper bottle to add 1-2 drops directly on the slide, on top of tissue ensuring the tissue is completely covered by mounting media.

* You can use a clean glass stick to distribute the mounting media evenly before placing the cover slide.

4. Carefully place cover slides over tissue and mounting media.

* Avoid trapping air bubbles underneath the cover slide.

5. Allow slides to fully dry in a horizontal position for 30-40 minutes.

Step 9: Data analysis and optimization

1. Analyze with a light microscope.

2. If unsatisfied with the results, perform further optimization following the guidelines below:

2.1 If weak or no staining is observed:

• Check whether your primary antibody was raised in mouse.

• Check whether the target is expressed in the tissue being analyzed at moderate to high levels. If that is not the case, refer to publications to include a positive control in your experiment.

• Increase the concentration of primary antibody (up to 100 μg/mL).

• Extend antigen retrieval time (up to 60 minutes).

• Try using an alternative antigen retrieval buffer.

If none of the recommended approaches work, it is possible that the primary antibody is not suitable for IHC.

2.2 If moderate to strong nonspecific signal is observed:

• Check whether the tissue was contaminated with microorganisms during the experiment. Confirm that little to no blood exists in your tissue sample before slicing.

• Try lowering the concentration of the primary antibody or shortening the primary antibody incubation time (down to 30 minutes).

• If this kit is applied to mouse tissue samples, background signal might be observed due to endogenous immunoglobulin being directly detected by the secondary antibody. To overcome this, try increasing the primary antibody concentration or shortening the secondary antibody incubation time. It is also highly recommended setting up a negative control without the primary antibody in this situation.

• Try shortening the antigen retrieval time (down to 15 minutes).

• Skip the quenching and/or signal enhancement steps.

• Use an alternative antigen retrieval buffer.

If none of the recommended approaches work, it is possible that the primary antibody is not suitable for IHC.

* **Important: DO NOT** dilute the secondary antibody using the Primary Antibody Diluent (Part NO. HC004) provided in this kit.