proteintech®
Antibodies | ELISA kits | Proteins

IHC*easy*

Ready-to-Use IHC Kit

IHC*easy* Contents

What's inside:

Antigen Retrieval Solution 50x

Wash Buffer 20x

Blocking Buffer 3% BSA

Primary Antibody

Secondary Antibody

Chromogen Solution A & B

Signal Enhancer

Counterstaining Reagent

Mounting Media

What's not included:

Xylene

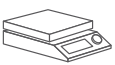
Ethanol

Double Distilled or Purified Water

Coplin Jars or Beakers

Slide Basket

Foil or Material to Cover Beaker



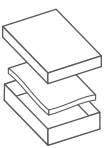
Heating Element

Slide Basket Container

Hydrophobic IHC Pen (Optional)

Thermometer

Wash Bottle



Wet Box

Microcentrifuge Tubes

Pipette

Cover Slips

IHC*easy* Information

Iconography:

Caution/Take note


Avoid/Beware

Perform action multiple times


Perform action for set time


Decrease temperature


Increase temperature


Add specified number of drops

Important Information:

Warning: Use proper protective equipment when working with xylenes. Xylenes can affect you when inhaled or upon contact with skin.

- Contact can irritate skin and eyes.
- Inhaling xylenes 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 when handling xylenes such as gloves, protective clothing, eye protection, and adequate ventilation.
- Dispose of xylenes and all kit waste properly.

Tips for using IHC*easy* Kits:
To maximize the use of the reagents in this kit, we recommend:

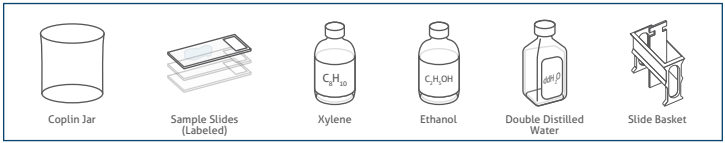
When using the dropper bottles, apply the recommended amount of drops onto the slide and use the tip of the dropper bottle to distribute the liquid evenly across the tissue section. If the tissue is not completely covered, add 1-2 additional drops of reagent and repeat.

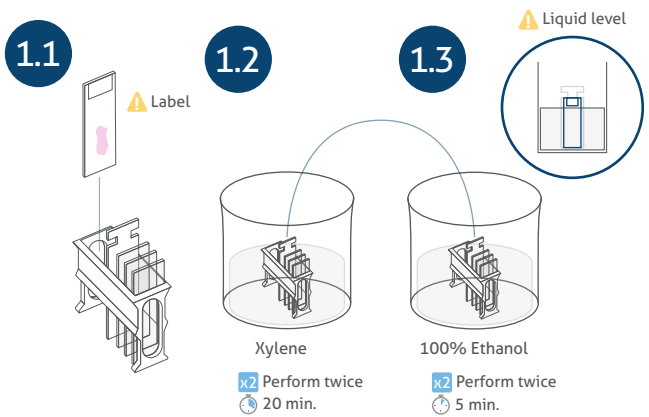
For the concentrated 50x antigen retrieval buffer and the 20x wash buffer, calculate how much of each buffer will be needed for the experiment and only prepare an appropriate amount of working solution. The working solution has a shorter shelf life than the concentrate.

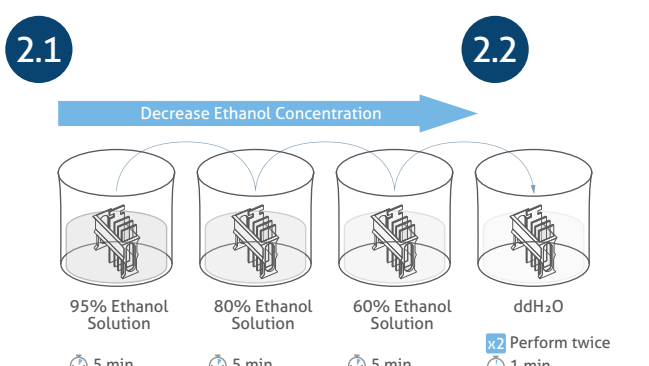
If a hydrophobic pen is not being used, keep slides as flat as possible to prevent the liquid from running off.

Step 0: Paraffin Removal & Rehydration

What you'll need:







Step 0: Paraffin Removal & Rehydration

Procedure:

1.1 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.

1.2 Immerse slides in xylene for 20 minutes. Repeat once using a separate container with fresh xylene.

1.3 Immerse slides in 100% ethanol tank for 5 minutes. Repeat once in fresh 100% ethanol.

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

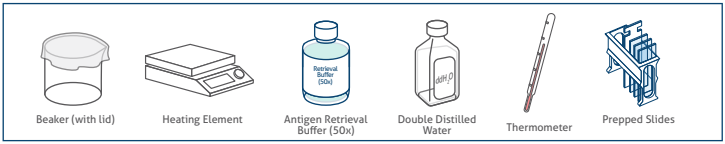
- 95% ethanol
- 80% ethanol
- 60% ethanol

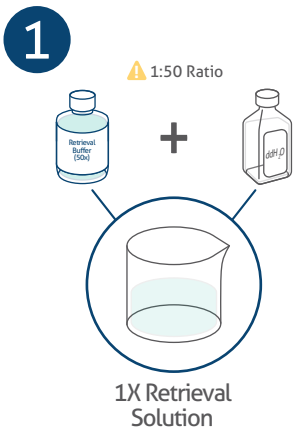
*TIP: antigen retrieval buffer can be prepared and heated during incubation period (See STEP 1 & 2 on following page)

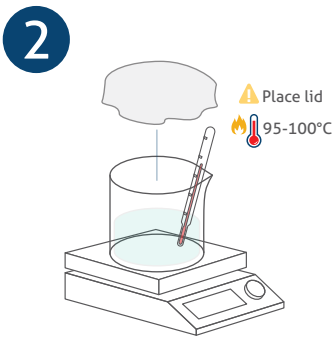
2.2 Rinse twice in dd$H_2O$for 1 minute each time. Use fresh ddH_2O for each wash.

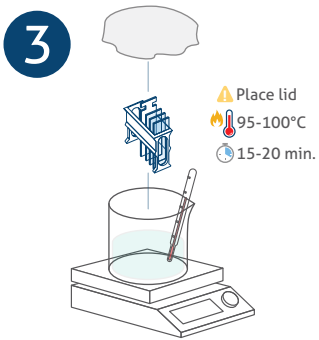
Step 1: Antigen Retrieval

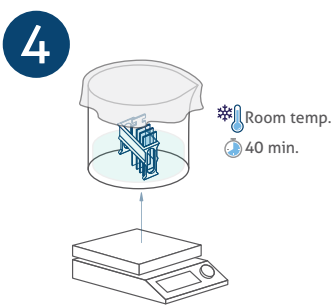
What you'll need:











Step 1: Antigen Retrieval

Procedure:

1. Use concentrated **50x Antigen Retrieval Buffer** to prepare a 1x solution by adding concentrated buffer to ddH_2O.

*500mL prepared 1x antigen retrieval buffer in a 1L beaker should be suitable for 1 or 2 baskets. Only prepare as much working solution as needed for the number of slides being stained.

2. Heat 1x buffer solution to 95˚–100˚C with a heating element.

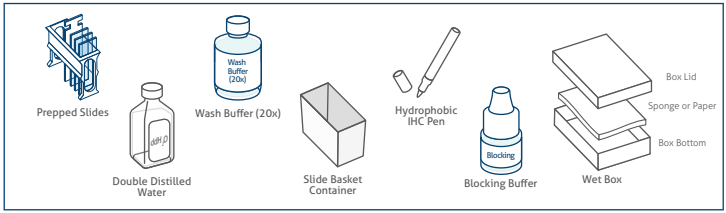
* Cover the beaker with a lid or foil to avoid vaporizing/vapor inhalation.

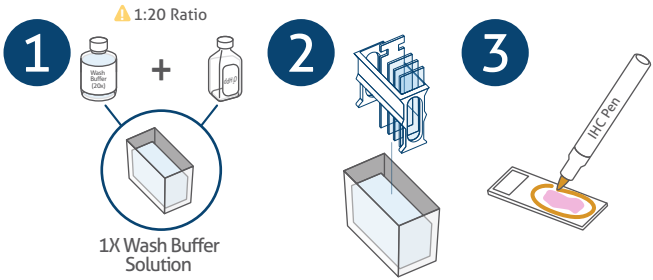
3. Place slides basket into heated antigen retrieval buffer. Maintain this temperature while incubating for 15-20 minutes.

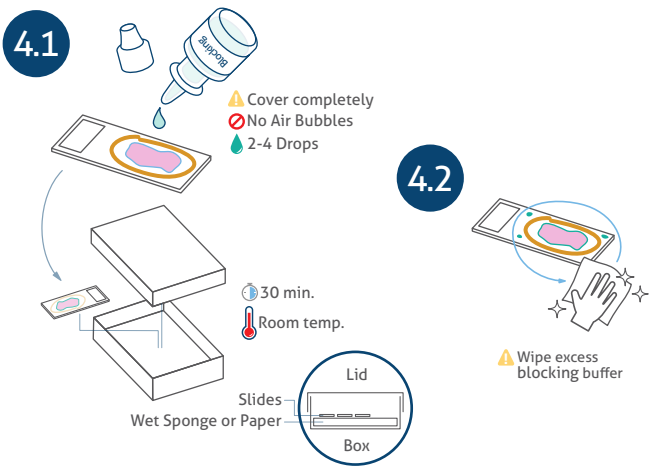
4. Remove the beaker from heat and let it cool to room temperature for 35–40 minutes.

Step 2: Blocking

What you'll need:







Step 2: Blocking

Procedure:

*Note: These kits have been validated for use without a quenching step. If you wish to include a quenching step on your samples to eliminate endogenous peroxidase activity, we recommend using 3% H_2O_2 (diluted in distilled water) for 10 minutes and then washing. This step is not required with the ready-to-use kit.

1. Use **20x Wash Buffer** to prepare a 1x solution by adding concentrated wash buffer to ddH_2O.

2. Wash slides by rinsing with ddH_2O and then briefly immersing in 100 mL **1x Wash Buffer**.

3. Use a hydrophobic IHC pen to draw circle on the glass slide around tissue section. (optional)

4.1 Use **Blocking Buffer** 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.

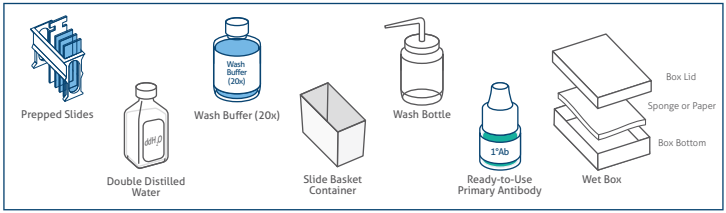
* TIP: Use the tip of the dropper bottle to distribute the liquid evenly covering the entire tissue section.

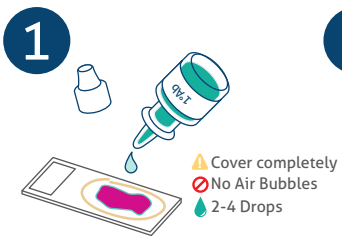
* Do NOT let the slides dry out.

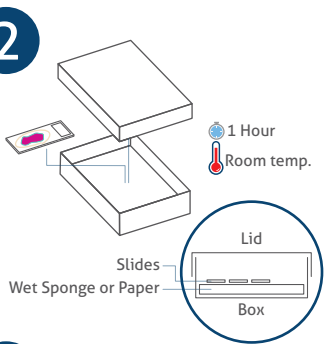
4.2 Drain blocking buffer off the slide and absorb any residual buffer around the tissue section.

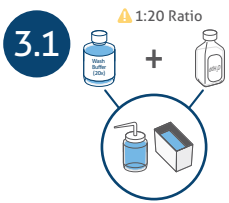
Step 3: Primary Antibody

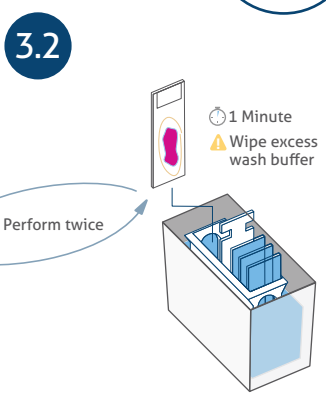
What you'll need:











Step 3: Primary Antibody

Procedure:

1. Use the ready-to-use **Primary Antibody** dropper bottle to add 2-4 drops directly on the slide, covering the entire tissue.

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

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

* Do NOT let the slides dry out.

3.1 Prepare a 1x wash buffer solution using the **20x Wash Buffer** and ddH_2O. Add prepared buffer to a wash bottle and coplin jar or beaker.

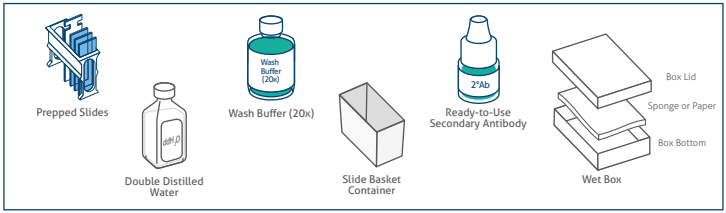
3.2 Use wash bottle to rinse primary antibody off slides. Then immerse slides in 1x wash buffer for 1 minute. Repeat the rinse and wash steps one more time; drain excess buffer and absorb any residual buffer around the tissue section.

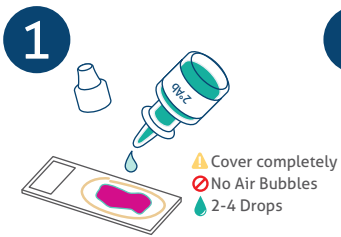
* If different primary antibodies are being used in the same experiment, wash in separate containers of wash buffer.

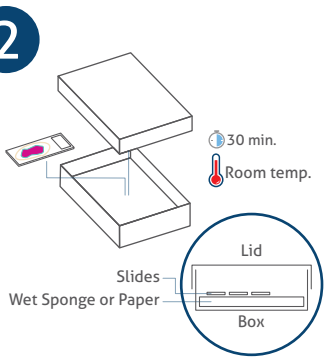
* Use fresh wash buffer for each wash.

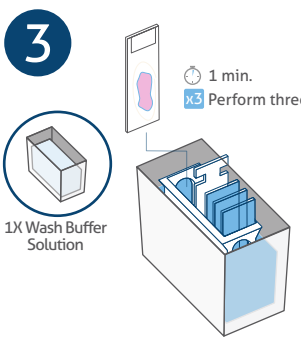
Step 4: Secondary Antibody

What you'll need:









Step 4: Secondary Antibody

Procedure:

1. Use the ready-to-use **Secondary Antibody** dropper bottle to add 2-4 drops directly on the slide, covering the entire tissue.

* 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.

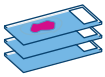
* Do NOT let the slides dry out.

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

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

Step 5: Signal Development

What you'll need:


Prepped Slides

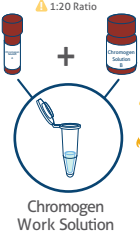

Chromogen Solution A & B


Microcentrifuge Tubes


Pipette


1X Wash Buffer (Wash Bottle)

1

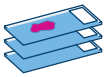


2

3

Step 6: Signal Enhancement

What you'll need:

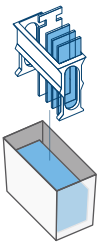

Prepped Slides


1X Wash Buffer (Slide Basket Container)

Signal Enhancer

1

2



Step 5: Signal Development

Procedure:

* Take out hematoxylin and bring to room temp ahead of counter staining step

1. In a tube, use pipette to prepare an appropriate volume of **Chromogen** in a microcentrifuge tube. Ratio of solutions A to B is 1:20.

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

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

3. Use wash bottle to rinse slides with 1x wash 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

Procedure:

1. Use the **Signal Enhancer** 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 wash buffer to rinse. Drain excess buffer off slides and absorb any residual buffer around the tissue section.

Step 7: Counterstain

What you'll need:


Prepped Slides

Counterstaining Reagent

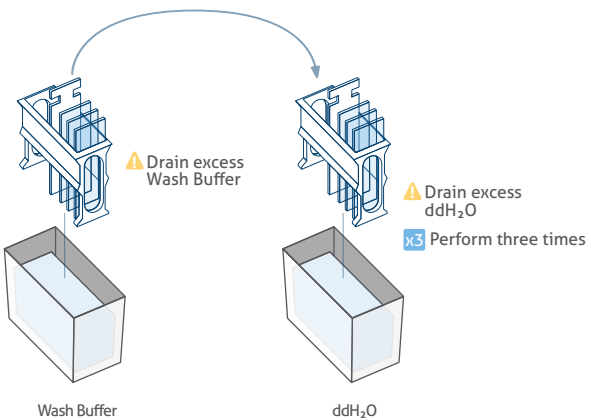

1X Wash Buffer


ddH_2O

1

2-4 Drops
2-3 min.

2



Step 7: Counterstain

Procedure:

1. Use the ready-to-use **Counterstaining Reagent** 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 wash 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

What you'll need:

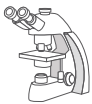

Prepped Slides

Xylene

Ethanol

Mounting Reagent

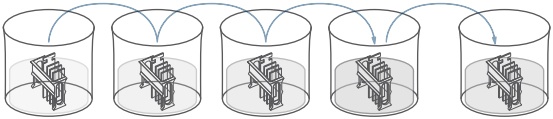

Cover Slips


Microscope

1.1

1.2

Increase Ethanol Concentration



60% Ethanol Solution — 5 min.

80% Ethanol Solution — 5 min.

95% Ethanol Solution — 5 min.

100% Ethanol — 5 min.

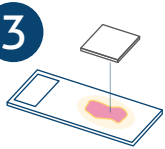
Xylene

- x2 Perform twice with fresh Xylene
- 5 min.
- Drain and absorb excess liquid
- Area must be well ventilated

2

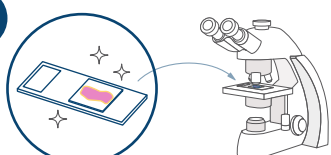
2-4 Drops

3



- No Air Bubbles
- 30–40min in ventilator

4



Completed IHC Slide

Step 8: Mounting

Procedure:

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

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

1.2 Immerse 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.

2. Use the mounting media reagent dropper bottle to add 1-2 drops directly to 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.

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

*Avoid trapping air bubbles underneath the cover slide

4. Allow slides to fully dry in a horizontal position for 30-40 minutes before observing with a light microscope.