

IHC Detect Kit for Rabbit Primary Antibody Data Sheet

Cat.NO.: PK10009

General Information:

Sample type: FFPE tissues

Assay type: Immunohistochemistry

Secondary antibody host species: Goat

Secondary antibody species specificity: Rabbit

Description:

The PK10009 kit is designed to facilitate signal detection during IHC analysis of FFPE tissue samples using primary antibodies raised in rabbits. The kit consists of Polymer HRP-Goat anti-Rabbit Secondary Antibody and ultra-sensitive DAB Chromogen for highly sensitive detection of rabbit primary antibodies. The kit also contains an optimized primary antibody dilution buffer that preserves primary antibody activity and helps lower non-specific background staining.

Note:

*If antigen retrieval buffer, counter staining reagent and mounting media are needed, please see our IHC Prep & Detect Kit for Rabbit Primary Antibody (Cat.NO. PK10017).

Components:

Part NO.	Component	Size
HC000	Quenching Buffer (RTU)	6 mL
HC004	Primary Antibody Diluent (RTU)	24 mL
HC005R	Polymer HRP-Goat anti-Rabbit Secondary Antibody	6 mL
HC006	Chromogen Component A	0.8mL
HC007	Chromogen Component B	8 mL

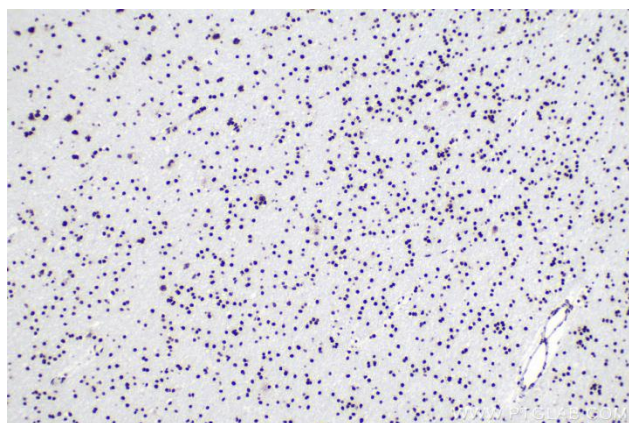
Size: 60 tests (may vary depending on tissue size)

Storage: All the reagents are stored at 2-8°C. The kit is stable for 6 months from the date of receipt.

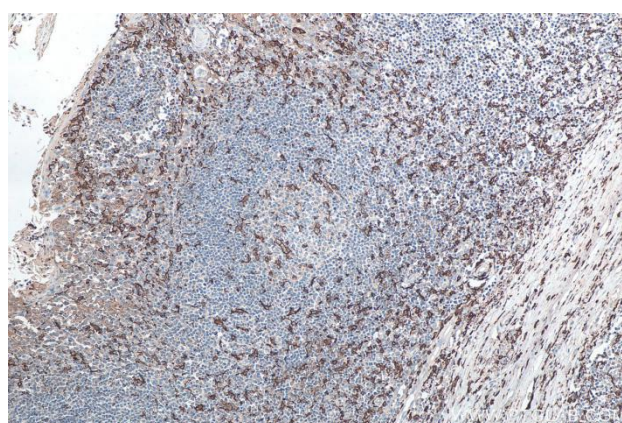
Application Note:

This kit is recommended for FFPE tissues and not for frozen sections.

Validation Data:



IHC analysis of human gliomas tissue using Proteintech's TDP43 rabbit recombinant antibody (80001-1-RR) and IHC Detect Kit for Rabbit Primary Antibody (PK10009).



IHC analysis of human tonsillitis tissue using Proteintech's IBA1 rabbit polyclonal antibody (10904-1-Ap) and IHC Detect Kit for Rabbit Primary Antibody (PK10009).

Recommended Protocol:

1. Prepare slides from tissues sections following routine methods. Deparaffinize tissues with xylene and re-hydrate using a decreasing ethanol gradient.
2. Perform antigen retrieval following the manufacturer's recommendations of the primary antibody.
3. Quenching (optional): 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.

3.1 Wash the slides with 1x wash buffer. Drain off the liquid on the slides and absorb any residual buffer around the tissue section (making the slides as dry as possible). Use a hydrophobic IHC pen to draw a circle on the glass slides around tissue sections (optional).

3.2 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 slides with 1x wash buffer after quenching.

4. Block the tissues with blocking buffer (e.g., 3% BSA in PBS) for 30 minutes at room temperature. Drain the liquid off the slides and absorb the blocking buffer around the tissue section.
5. Use the Primary Antibody Diluent (Part NO. HC004) in the kit to dilute your primary antibody (ONLY primary antibodies raised in rabbits should be used with this kit). Apply 80-200 μ L (depending on the size of the tissue) of the diluted primary antibody to the slide, covering the entire tissue. Incubate at room temperature in a wet box with a lid for 1 hour.
6. Wash the slides three times with 1x wash buffer following primary antibody incubation. Drain the liquid off the slides and absorb any residual buffer around the tissue section. Use the ready-to-use Polymer HRP-Goat anti- Rabbit Secondary Antibody (Part NO. HC005R) dropper bottle to add 2-4 drops directly on the slide, covering the entire tissue. Incubate at room temperature in a wet box with a lid for 30 minutes.
7. Wash the slides three times with 1x wash buffer following secondary antibody incubation. Drain the liquid off the slides and absorb any residual buffer around the tissue section. Use pipette to prepare an appropriate volume of DAB 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 μ L/slide is needed.
Use pipette to add enough DAB 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 wash buffer.
8. Perform counter staining with hematoxylin.
9. Wash the slides with wash buffer or pure water. Dehydrate tissues using an increasing ethanol gradient followed by xylene. Mount the slides with cover slides and analyze with a light microscope once they are fully dried.