

Protease K Antigen Retrieval Buffer

Data Sheet

Cat.NO.: PR30014

General Information:

Sample type: FFPE tissues

Assay type: Immunohistochemistry

Concentration: 2.0mg/mL (Stock)

pH (1x): 7.4

Description:

PR30014 is a protease K based solution that can be used for performing protease-induced epitope retrieval (PIER) in immunohistochemistry applications. While treating tissues with formalin and xylene are necessary steps for fixation and deparaffinization in an immunohistochemistry workflow, such treatments often result in protein cross-linking leading to the masking of antigenic sites and the subsequent inhibition of antigen-antibody interactions. Antigen retrieval helps restore the structure of such proteins by breaking the cross-links and unmasking the antigenic sites, thereby making them more accessible to antibodies.

Size:

0.2mL Protease K 100x Stock + 20mL Diluent

Shipping & Storage:

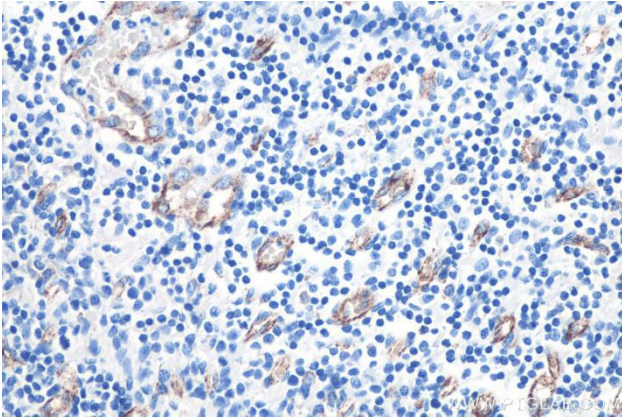
Shipped at -20°C. Store Protease K 100x Stock at -20°C and Diluent at 4°C or -20°C upon receipt. The product is stable for 6 months from the date of receipt.

Application Note:

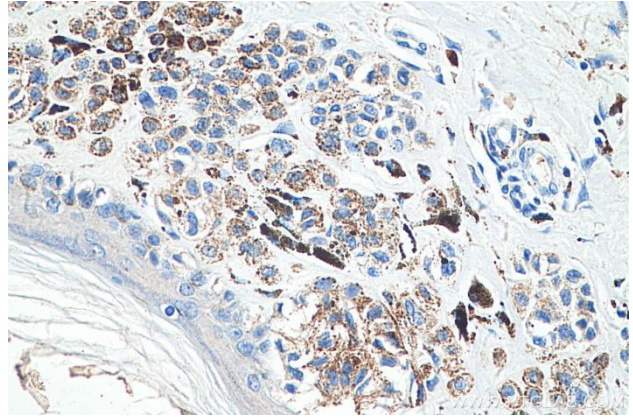
1. Dilute to 1x before use.

2. Calculate how much antigen retrieval buffer will be needed for the experiment to prepare an appropriate amount of 1x working solution. The working solution has a shorter shelf life than the concentrate.

Validation Data:



IHC analysis of human tonsillitis tissue with Proteintech's VWF rabbit polyclonal antibody (27186-1-AP). Protease-induced epitope retrieval was performed by incubating at 37°C for 5 minutes in Protease K Antigen Retrieval Buffer (PR30014).



IHC analysis of human malignant melanoma tissue with Proteintech's ALPP mouse monoclonal antibody (60294-1-1g). Protease-induced epitope retrieval was performed by incubating at 37°C for 15 minutes in Protease K Antigen Retrieval Buffer (PR30014).

Recommended Protocol:

- * Thaw the Protease K 100x Stock and the Diluent (if stored at -20°C) before use.
1. Prepare slides from tissues sections following routine methods. Deparaffinize tissues with xylene and re-hydrate using a decreasing ethanol gradient.
 2. Wash the slides with washing buffer. Drain off the liquid from the slides and absorb any residual liquid around the tissue. Circle the tissue with a hydrophobic IHC pen (optional).
 3. Use Protease K 100x Stock to prepare a 1x working solution by adding the concentrated buffer to the diluent. For example, to make 10mL of 1x working solution, dilute 0.1mL Protease K 100x Stock with 9.9mL Diluent.
 4. Apply 100-150 μ L of the 1x working solution to the slides and incubate at 37°C for 5-15 minutes in a wet box. Make sure that the retrieval buffer covers the whole tissue. If the tissue is too large, add an additional 50-100 μ L of the 1x working solution.
 5. Store the remaining undiluted Protease K 100x Stock back at -20°C.
 6. Perform subsequent steps of quenching, blocking, primary and secondary antibody incubation, signal development, counter staining and mounting before analyzing the slides.