Technical Data Sheet

Retrievagen A (pH 6.0)

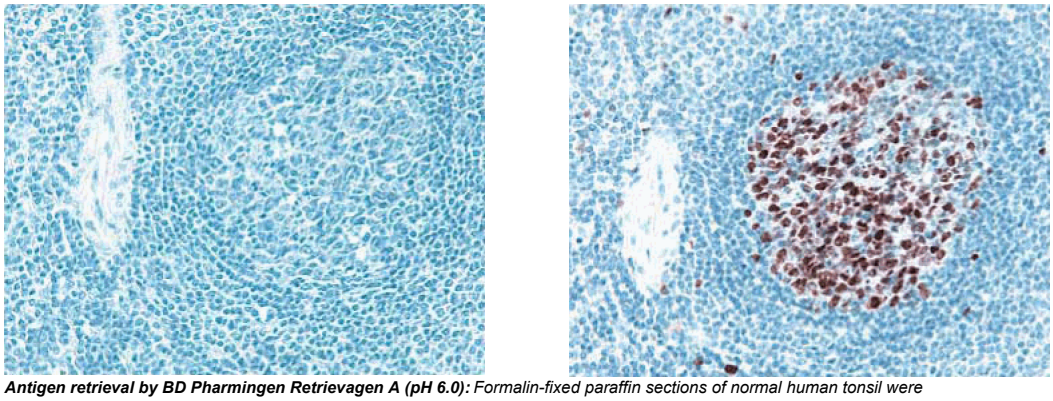
Product Information

Material Number:	**550524**
Component:	**51-7540KC**
Description:	Retrievagen A Solution 1
Size:	25 ml (1 ea)
Component:	**51-7541KC**
Description:	Retrievagen A Solution 2
Size:	100 ml (1 ea)

Description

BD Retrievagen A (pH 6.0) is an antigen retrieval system that is recommended for Zinc-fixed and Formalin-fixed paraffin embedded tissue sections that show sub-optimal or no reactivity with antibodies for immunohistochemical staining. 10% neutral buffered formalin fixes the tissue by cross-linking proteins that may cause a conformational change or "masking" of the antigenic epitope leading to reduced or absent antibody reactivity. The BD Retrievagen A (pH 6.0) is designed to unmask the epitope and cause the enhancement of antibody staining. The proposed mechanism by which most of the antigen retrieval systems work is through breaking the cross-linking bonds formed between the protein molecules.

It is important to realize that in spite of antigen retrieval, some antibodies do not stain on formalin-fixed paraffin embedded sections. In these situations fixing the tissue in a less harsh fixative like the IHC zinc fixative developed by BD Pharmingen (Cat. No. 550523) may be helpful. In many cases our zinc-fixative preserves the epitope leading to good antibody staining at the same time depicting good tissue morphology. However, there are a few antigens that are not preserved by any gentle fixation, and in these situations use of fresh frozen tissue for immunohistochemical staining is the only alternative.



Antigen retrieval by BD Pharmingen Retrievagen A (pH 6.0): *Formalin-fixed paraffin sections of normal human tonsil were either untreated (left panel) or pre-treated with BD Retrievagen A (pH 6.0) (right panel) nd then stained with the anti-Ki-67 antibody. Note the absence of antibody staining in the untreated versus the panel where antigen retrieval solution was used.*

Preparation and Storage

Store undiluted at 4°C.

Application Notes

Recommended Assay Procedure:

BD Pharmingen Retrievagen A (pH 6.0) is provided as two separate solutions that need to be mixed. Take 18 ml of BD Retrievagen A solution 1 and 82 ml of BD Retrievagen solution 2 and bring up the volume to 1 litre with distilled water. The pH of the final working solution should be around 6.0. This working solution can be stored at 4° C for up to a month. Antigen retrieval protocol is performed after deparaffinizing the slides as outlined below.

1. Deparaffinize slides (after drying thoroughly overnight at RT) in 2 changes of xylene for 10 min each.
2. Wash slides in 100% alcohol twice for 2 min each.

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3. Block endogenous peroxidase activity by incubating for 10 min in 3% H2O2. Rinse 2-3 times in water.
4. Place slides in a plastic coplin jar or staining dish with the BD Retrievagen A working solution and heat to 193°F in a microwave.
5. Mix the solution and incubate the slides at the desired temperature of 193°F for 10 min.
6. Remove the staining dish, cover and allow the slides to slowly cool to room temperature for 20 min.
7. Wash slides with 2-3 changes of water and continue with the blocking with normal serum and application of the primary antibody.

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Chan JK. Advances in immunohistochemical techniques: toward making things simpler, cheaper, more sensitive, and more reproducible. *Adv Anat Pathol.* 1998; 5(5):314-325.(Biology)

Pileri SA, Roncador G, Ceccarelli C, et al. Antigen retrieval techniques in immunohistochemistry: comparison of different methods. *J Pathol.* 1997; 183(1):116-123. (Biology)

Shi SR, Key ME, Kalra KL. Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem.* 1991; 39(6):741-748.(Biology)