

Technical Data Sheet

BD Retrieval B (pH 9.5)

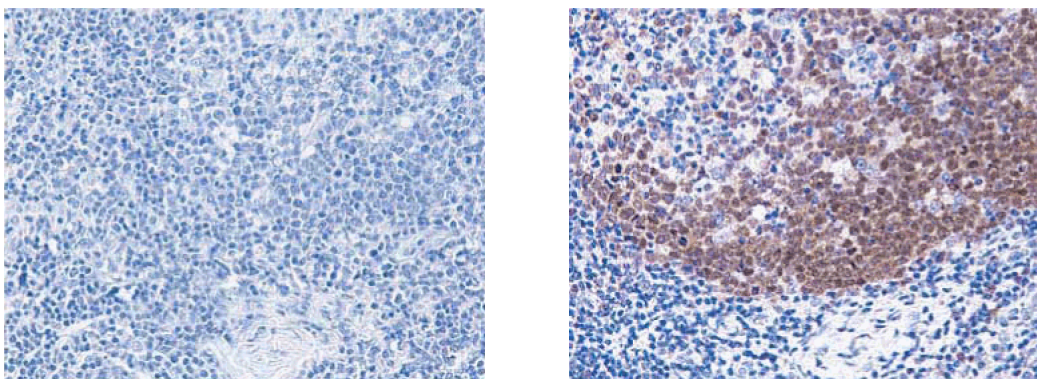
Product Information

Material Number: 550527
Size: 250 ml

Description

BD Retrieval B (pH 9.5) 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 Retrieval B (pH 9.5) 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 in the fixed tissue sections.

It is important to realize that in spite of antigen retrieval, some antibodies do not stain on formalin-fixed paraffin 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. 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 Zinc-fixation either and in these situations use of fresh frozen tissue for immunohistochemical staining is the only alternative.



Antigen retrieval by BD Retrieval B (pH 9.5): Formalin-fixed paraffin sections of normal human tonsil were either untreated (left panel) or pre-treated with BD Retrieval B (pH 9.5) (right panel) and then stained with the anti-human topoisomerase II 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

Application

Immunohistochemistry-paraffin	Routinely Tested
Immunohistochemistry-zinc-fixed	Routinely Tested

Recommended Assay Procedure:

BD Retrieval B (pH 9.5) is provided as a ready to use solution. Antigen retrieval procedure is performed after deparaffinizing the slides as outlined below.

1. Deparaffinize slides (after drying thoroughly overnight at room temperature) in 2 changes of xylene for 10 min each.
2. Wash slides in 100% alcohol twice for 2 min each.
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 Retrieval B solution and heat to 193° F in a microwave.

BD Biosciences

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5. Mix the solution and incubate the slides at the desired temperature of 193° F for 5 min.
6. Remove the staining dish, cover and allow the slides to slowly cool to room temperature for 10 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/pharming/en/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)