

The synergistic relationship of enzyme and HIER - For strong, specific staining of difficult antibodies

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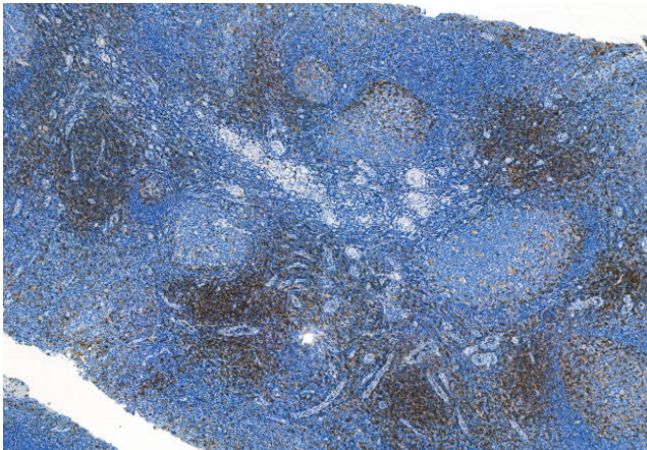
With the increased use of heat-induced epitope retrieval (HIER) for formalin-fixed paraffin-embedded (FFPE) tissues on automated immunohistochemistry (IHC) instruments, enzymatic digestion use has decreased. Automated instruments often lack the capacity to exceed 100°C, resulting in less than optimal results compared to offline antigen retrieval methods. The use of enzymes alone for epitope retrieval lacks in comparison to HIER as higher concentrations and incubation times are needed to produce appropriate expression but run the risk of tissue over-digestion. Using a synergistic relationship of HIER and enzymatic digestion, automated instruments can make staining equivalent to offline pressured HIER methods.

Using automated and semi-automated IHC instrumentation, Desmoglein 3, MSH6, and CD4 slides were stained with and without a weak post-retrieval enzymatic digestion utilizing Pronase and Trypsin. With the use of Pronase, Desmoglein 3 and CD4 exhibited significant increase in staining. Trypsin increased the staining of MSH6 by one grade increase. All antibodies using this synergistic relationship not only enhanced staining intensity but exhibited increased sensitivity when compared to the control with offline HIER using Biocare Medical's Decloaking Chamber™ NxGen.

A synergistic relationship between HIER and enzyme digestion was easily achieved without compromising tissue integrity. The addition of enzymatic digestion after HIER proved to be a superior method that not only increased staining intensity but allowed for the detection of positive cells that might have otherwise been missed during review. This combination pretreatment approach is ideal for problematic or difficult antibodies stained on automated IHC platforms with a less than optimal online HIER.

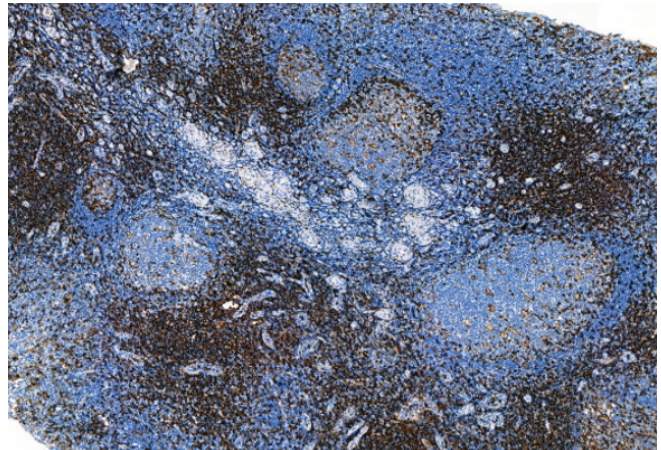
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Without Enzymatic Digestion



CD4 (RM) in tris-based buffer without post-retrieval enzymatic digestion. Low staining intensity and decreased sensitivity can be seen.

With Enzymatic Digestion



CD4 (RM) in tris-based buffer with post-retrieval enzymatic digestion with high increase in staining intensity.

To learn more about the Biocare's offering for your lab, please contact us anytime at 800-799-9499 or visit our website at www.biocare.net.