WHITEPAPER

Biocare Basics:
Biotin Based Detection

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Biocare Basics: Biotin Based Detection

Immunohistochemistry (IHC) revolves around the mechanism of bringing enzyme to the site of an antigen of interest. The conjugated enzyme reacts with the chromogen to generate a colored substrate in order for the antigen location to be visible under a light microscope. IHC detection types are differentiated by the method they use to bind enzyme to the antigen site. One common detection type is Avidin-Biotin detection which utilizes a biotinylated secondary antibody and enzyme-linked avidin or streptavidin for IHC staining.

Avidin is a naturally occurring glycoprotein found in egg albumin.[4] Biotin, also known as vitamin B7, can be found in all living cells.[4] Together, they bind rapidly, forming one of the strongest non-covalent bonds in nature.[4] The binding affinity and extreme stability of biotin with avidin and its homologs have made avidin-biotin binding a favored mechanism in IHC staining applications for decades.

At the start of the IHC protocol, a peroxidase block is first applied to block endogenous peroxidase enzyme in the sample. In the case of a biotin-based detection system, a biotin block must then also be applied in order to block any endogenous biotin.[1] These two blocking steps prevent naturally occurring peroxidase and biotin in the tissue from cross-reacting with the detection system and causing nonspecific background staining.

The primary antibody is applied next, followed by a secondary antibody. In biotin-based detections, this secondary antibody is conjugated to a biotin molecule in a process known as "biotinylation."[1] The biotinylated secondary binds the primary antibody, making biotin present and available at the antigen site for avidin or its homologs to bind to.

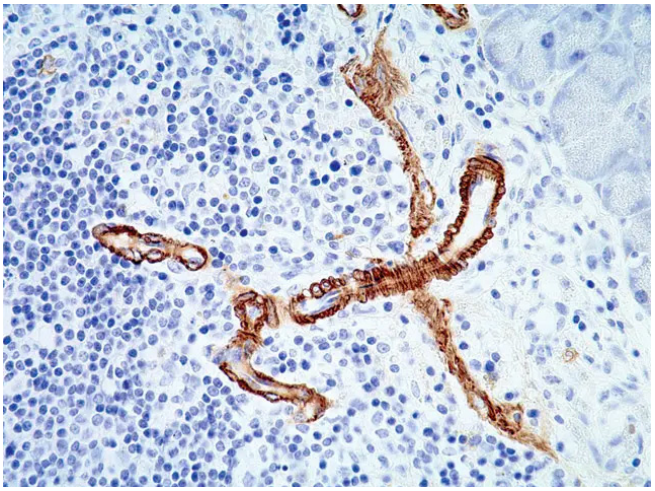
Next, an enzyme-linked avidin or avidin homolog is applied to bind to the biotin. Presently, the homolog streptavidin is favored over avidin in IHC detection. Like avidin, streptavidin binds four biotin molecules and is highly stable.[3] However, unlike avidin, streptavidin is uncharged at neutral pH and lacks carbohydrate side chains which make it less prone to background staining when compared to avidin.[3]

Additionally, streptavidin provides a significant increase in sensitivity, perhaps as a result of less steric hindrance.[1]

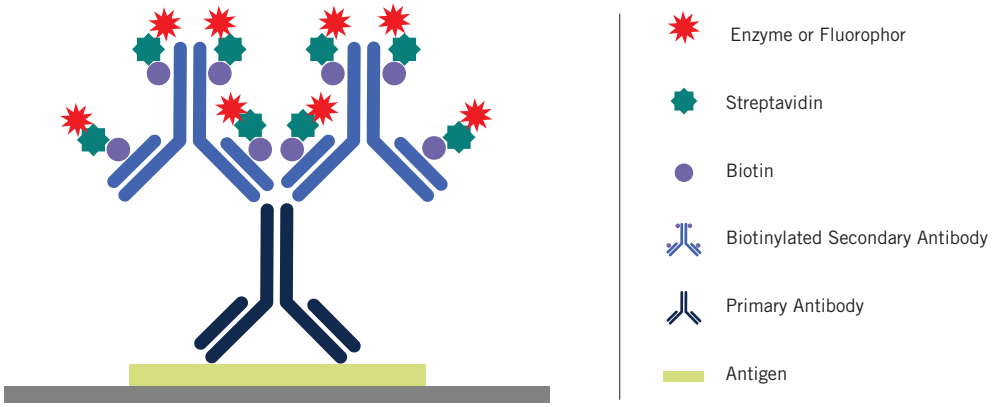
Finally, a chromogen is applied that will react with the enzyme present in the detection in order to generate a colored substrate that can be visualized under a microscope.

Biocare Medical's Starr Trek detection system and 4plus detection system utilize streptavidin staining technology to provide the cleanest, clearest biotin-based IHC staining possible. These detections feature a long spacer arm between the biotin and the secondary antibody, which provides a significant increase in sensitivity when coupled with streptavidin conjugates.

Both Starr Trek and 4plus detection systems have been designed for reliable, cost-effective, biotin-based detection, providing a high level of sensitivity. These detection systems can be used with mouse and rabbit primary antibodies and can be used with either horseradish peroxidase or alkaline phosphatase enzymes.



Mouse Smooth Muscle Actin with B- and T-cells stained with Biocare 4Plus Detection



To learn more about cost-effective biotin-based detection options for your lab, please visit us at biocare.net or call 1-800-799-9499.

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2. "Detection 4plus", Biocare Medical, https://biocare.net/detection-four-plus/
3. Jain, A., & Cheng, K. (2017). The principles and applications of avidin-based nanoparticles in drug delivery and diagnosis. Journal of controlled release : official journal of the Controlled Release Society, 245, 27–40. https://doi.org/10.1016/j.jconrel.2016.11.016
4. McMahon, R. J., Haugland, R. P., & You, W. W. (2010). Coupling of Antibodies with Biotin. In Avidin-biotin interactions: Methods and applications (pp. 13–22). essay, Humana.