WHITEPAPER

Understanding Chromogens vs. Dyes in Immunohistochemistry

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The ultimate goal of any immunohistochemistry (IHC) procedure is applying color to otherwise colorless cellular features, also known as staining. IHC staining allows slides to be visualized under a microscope for diagnostic analysis or research purposes and is mainly accomplished through the use of substances called chromogens. The purpose of a standard IHC protocol is to deposit colored chromogen product at the antigen binding sites to produce the stain. Therefore, understanding the concept of a chromogen and its mechanism of action is key to understanding the IHC protocol itself.

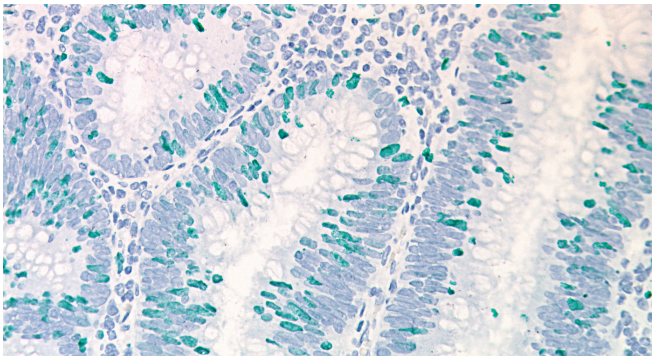
Whenever staining is discussed, the image that likely comes to mind is that of dyes. From hair to clothing, food, and arts and crafts, dyes are a common, familiar concept to most people. However, while the stain produced by a dye may be visually similar to that of a chromogen, their mechanism of action is quite different.

Dyes are soluble in water or an organic solvent and work by binding to things that they have a molecular affinity for.[2] In other words, the preexisting, colored compounds in the dye stick to the object being stained, effectively transferring their color. A well-known example of this in the lab is the reagent hematoxylin. Hematoxylin is a naturally derived dye that is produced from the heartwood extract of the logwood tree Haematoxylum campechianum.[5] When applied to cells, the positively charged hematoxylin compounds are attracted to the negatively charged chromatin in the cell nuclei, binding them and making them appear blue.[4]

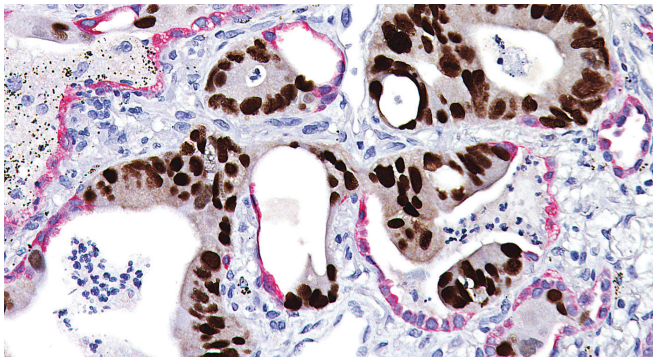
In contrast, a chromogen is a colorless chemical compound that is only converted to a colored compound through a chemical reaction. In IHC, this reaction occurs by the action of enzymes. In the IHC protocol, enzymes are directly or indirectly attached to the antibody-antigen binding site in order to catalyze the conversion of chromogen substrates into different colored products.[3] Common chromogens include 3,3'-diaminobenzidine tetrahydrochloride (DAB), which reacts with the enzyme horseradish peroxidase (HRP) to produce a brown end product, or Fast Red, which reacts with the enzyme alkaline phosphatase (AP) to produce a red end product.[3] A variety of chromogens are available to provide an array of color options.

Since IHC staining relies on bringing the colorless chromogen into contact with its corresponding enzyme, the IHC protocol must result in the appropriate enzyme being present at the sites that should be stained. It is the function of the IHC detection reagents to deposit the enzyme. Excess enzymes in the surrounding tissue must also be blocked to prevent nonspecific background staining, which is performed by applying blocking reagents prior to the antibody steps.

Biocare Medical offers a wide range of blocking reagents, detection kits, and chromogens for both manual and automated staining. To determine which is right for you, please visit our website at biocare.net or feel free to call our Technical Support team at 1-800-799-9499 for product advice based on your laboratory's needs.



Colon cancer stained for Ki-67 with Biocare's Vina Green HRP chromogen



Metastatic colon cancer in lung tissue stained for CDX2 and CK7 in Biocare's DAB HRP chromogen and Fast Red AP chromogen

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