

# Equivalent Terms: Peroxidase Blocking in IHC

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The blocking steps of an immunohistochemistry (IHC) protocol are significant factors in the quality and clarity of IHC staining. Endogenous enzymes in the tissue must be prevented from interfering with the enzymes of the detection systems, and stray attractive charges must be neutralized to mitigate background staining. In the IHC protocol, the endogenous peroxidase in the sample is the first element to be blocked. This step may be referred to by different names.

The primary blocking step in an IHC protocol is the block of endogenous peroxidase enzyme in the tissue. Peroxidase enzymes are present in almost all living organisms.<sup>2</sup> In mammals, different peroxidases are distributed throughout tissues and their cells such as erythrocytes, granulocytes, and neurons.<sup>1,2</sup> However, peroxidase (specifically, horseradish peroxidase or HRP) has also become the standard enzyme marker in IHC detection due to its stability and chromogen clarity.<sup>1</sup> This creates a risk of false positives if preexisting endogenous peroxidase in the tissue sample is left to react with the chromogen during the detection step.

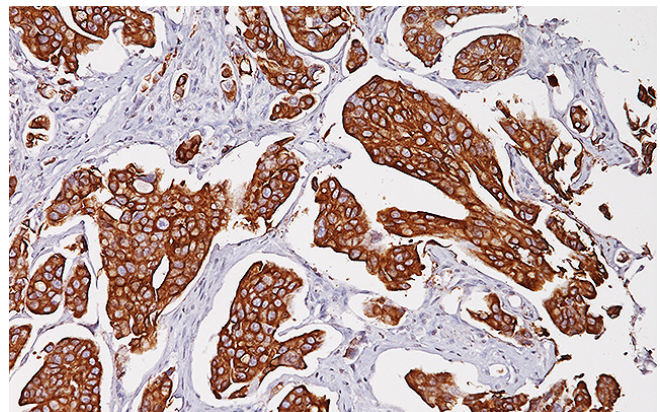
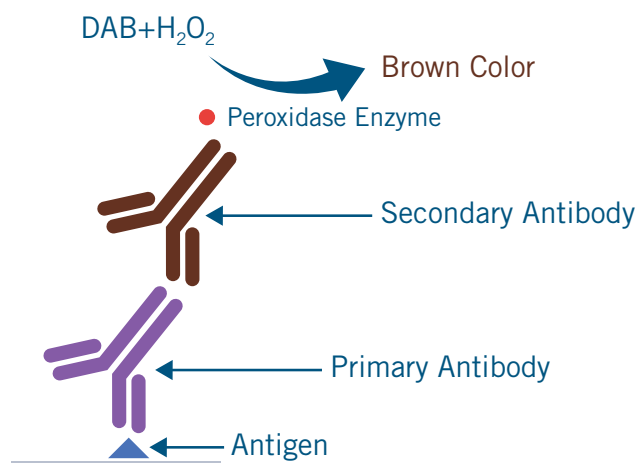
To block the endogenous peroxidase and help prevent false positives, an oxidative agent is used to “quench” the enzyme in the sample. Most commonly, this is done using hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>).<sup>1</sup> However, other oxidants may also be used to achieve the same effect.<sup>1</sup>

Blocking endogenous peroxidase in the sample is most commonly called the “peroxidase block,” which refers to the enzyme that is being blocked. On the other hand, laboratory technicians may also refer to it as a “peroxide block” if the block is being performed using hydrogen peroxide. In this case, the name refers to the solution that is being used to block the enzyme.

Biocare Medical offers both hydrogen peroxide based and non-hydrogen peroxide based blocking solutions to block endogenous peroxidase. Peroxidized 1 is a highly stable form of hydrogen peroxide that is very effective for blocking non-specific peroxidase staining. It is non-flammable, safer and less toxic when compared to hydrogen peroxide/methanol formulations.

PeroxAbolish is a novel formula that is a non-hydrogen peroxide blocking reagent that is safe and extremely gentle on tissues and cells. It is highly effective in quenching endogenous peroxidase and can be used on formalin-fixed paraffin-embedded tissues, cell culture, blood smears, cell preparations and frozen sections. When using paraffin-embedded formalin-fixed tissues, PeroxAbolish is extremely effective after antigen retrieval methods. Both blocking reagents can be used on automated staining systems.

### IHC DAB Diagram



Bladder cancer stained with Uroplakin II antibody

To learn more about blocking reagents at Biocare, please visit us at [biocare.net](http://biocare.net) or call 1-800-799-9499.

1. Bussolati, Gianni; Radulescu, Razvan Tudor (2011). Blocking Endogenous Peroxidases in Immunohistochemistry. *Applied Immunohistochemistry & Molecular Morphology*, 19(5), 484–. doi:10.1097/pai.0b013e318219a6e6
2. Khan, A. A., Rahmani, A. H., Aldebasi, Y. H., & Aly, S. M. (2014). Biochemical and pathological studies on peroxidases -an updated review. *Global journal of health science*, 6(5), 87–98. <https://doi.org/10.5539/gjhs.v6n5p87>