Dako
Dual Endogenous Enzyme Block

Code S2003
Ready-to-use

Intended use
For In Vitro diagnostic use.

This product is intended for use in peroxidase- as well as alkaline phosphatase-based Immunohistochemical (IHC) staining procedures on cell preparations, frozen tissue sections, and formalin-fixed paraffin-embedded tissue sections.

Reagents provided
Code S2003
Dual Endogenous Enzyme Block is available in the following sizes:

15 mL for manual staining
10x11 mL packaged for use with the Dako Autostainer

Precautions
1. For professional users.
2. As with any product derived from biological sources, proper handling procedures should be used.
3. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
4. Unused solution should be disposed of according to local, State and Federal regulations.

Storage
Store at 2–8°C.

Reagent preparation
Ready-to-use. Equilibrate to room temperature prior to use.

Procedure
Specimens are incubated with Dual Endogenous Enzyme Block for 10 minutes at room temperature followed by a rinse with a suitable wash buffer such as Tris buffer, PBS or TBS before application of primary antibody.

Results
In IHC staining procedures endogenous activity of peroxidase, pseudoperoxidase and alkaline phosphatase is frequently observed. Background staining that is not related to the immunospecific reaction may occur with certain molecules.

Molecules most commonly affected by peroxidase are hemoproteins such as hemoglobin in red blood cells, myoglobin in muscle cells, cytochrome in granulocytes, and monocytes, as well as catalases in the liver and kidney.1

Endogenous AP activity is strongly present on a number of cells and tissues including epithelium of the bladder, the lamina propria of ovary, kidney and salivary gland.2 Intestinal AP is commonly found on the brush borders of epithelial cells of the small intestine.3 Human placental AP is normally produced by the microvilli of the syncytiotrophoblast of placenta.4 In cell smear preparations, AP is present in neutrophilic segmented cells, neutrophilic band cells, and neutrophilic metamyelocytes. Alkaline phosphatase may also be found in leukemic cells of chronic and acute granulocytic leukemia, and in some neutrophilic leukemoid reaction.

In the evaluation of tissue specimens by IHC using HRP and AP methods, the presence of endogenous peroxidase and AP can often obscure the specific staining of the target antigen. Endogenous peroxidase and AP activity can be inhibited by the use of Dual Endogenous Enzyme Block which will suppress endogenous peroxidase, pseudoperoxidase, and alkaline phosphatase in cell preparations, frozen tissue sections, and paraffin-embedded tissue sections.

Specimens
Frozen tissue sections and cell preparations such as smears of peripheral blood, bone marrow, and other bodily fluids containing a large number of hematopoietic cells can be used. For frozen tissue sections, fixation in acetone is recommended. For smears of blood and bone marrow, fixation in acetone/methanol is recommended.

Applications
This product will suppress nonspecific staining due to endogenous peroxidase and pseudoperoxidase activity in Peroxidase-based IHC staining procedures. This product will also suppress nonspecific staining due to endogenous AP activity in AP-based IHC staining procedures.

Limitations
Specimen staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, or sectioning may produce artifacts, antibody trapping, or false positive results. False-positive staining may also be caused by cross-reactivity or nonspecific reaction with necrotic or degenerated cells. Lysis of erythrocytes may occur when using this product on blood smears and bone marrow smears.
References

2. Ponder BA and Wilkinson MM. Inhibition of endogenous tissue alkaline phosphatase with the use of alkaline phosphatase conjugates in immunohistochemistry. J Histochem Cytochem 1981; 29(8):981