Product Datasheet

Immuno HRP-DAB, Anti-Chicken IgY (H+L) (orb2652847)

Catalog Number	orb2652847
Category	Tools
Description	Immunohistochemistry (IHC)/Immunocytochemistry (ICC) is the localization of antigens by the use of antigens in tissue sections/cells by the use of labeled antibodies as specific reagents through antigen-antibody interactions that are visualized by a marker such as fluorescent dye, enzyme, radioactive element or colloidal gold. Several IHC techniques are commonly used: labeled biotin secondary antibody streptavidin-peroxidase (LBSASP), HRP anti-HRP, ABC, catalyzed signal amplification, polymer system and others, to detect antigens on tissue and cell. In this kit the first layer is unlabeled primary antibody, the second layer is biotinylated secondary antibody, the third layer is Enzyme-Streptavidin conjugate (HRP-Streptavidin) to replace the complex of avidin-biotin peroxidase. The enzyme is then visualized by application of the substrate chromogen solution to produce different colorimetric end products.
Tested applications	ICC, IHC

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Application notes	To prepare the DAB Chromogen reagent, add two drops of DAB Chromogen to one milliliter of DAB Buffer Substrate in a test tube and mix well. This ready-to-use reagent remains stable for six hours. The ratio of buffer substrate to chromogen is 40:1, allowing for flexible volume preparation. For IHC and ICC procedures, first deparaffinize and hydrate tissue sections through clearing agents and graded alcohols. Wash two to three times with distilled or deionized water. Incubate sections or cell smears in Endoblocker for 5 to 10 minutes at room temperature or 37°C. If an antigen retriever is required, it should be applied at this stage according to the primary antibody protocol. Wash the slide three to five times with PBS Tris saline or washing buffer. Incubate sections in Protein blocking solution for 10 minutes at room temperature or 37°C, then wash once with PBS. Incubate with the primary antibody for 20 to 30 minutes at room temperature or 37°C and wash five to seven times with PBS. Incubate with biotinylated secondary antibody for 15 minutes at room temperature or 37°C, followed by five to seven washes with buffer. Ensure all buffers and reagents are free of sodium azide to prevent peroxidase inactivation. Incubate with Streptavidin-Peroxidase reagent for 10 minutes at room temperature or 37°C. Wash the slide five to seven times with PBS, then two to three times with deionized or distilled water. Incubate with DAB reagent for 5 to 10 minutes at room temperature or 37°C. Wash five to seven times with distilled or deionized water. Apply hematoxylin counterstain for 30 to 60 seconds. Wash with tap water, distilled water, and then PBS buffer, maintaining the slide in buffer for 2 to 3 minutes until the hematoxylin transitions from purple to blue. Perform a final wash with distilled or deionized water before mounting the slide with aqueous or organic mounting medium.
Storage	2-8 °C, Do Not Freeze
Note	For research use only
Expiration Date	12 months from date of receipt.

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