Product Datasheet

Immuno HRP-AEC, Anti-Chicken IgY (H+L) (orb2652850)

Catalog Number	orb2652850
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

Biorbyt Ltd.
7 Signet Court, Swann Road
Cambridge
CB5 8LA
United Kingdom
Email: info@biorbyt.com, support@biorbyt.com
Phone: +44 (0)1223 859353 | Fax: +1 (415) 651-8558

Biorbyt LLC
68 TW Alexander Drive
Research Triangle Park
Durham
NC 27713-2847
United States
Email: info@biorbyt.com, support@biorbyt.com
Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

Application notes	To prepare Chromogen reagent 6, add two drops of reagent B buffer to 5 milliliters of distilled or deionized water in a test tube and mix well. Add one drop of reagent C chromogen and mix well, followed by one drop of reagent S substrate and a final mix. This ready-to-use reagent remains stable for several hours. For IHC and ICC procedures, first deparaffinize and hydrate tissue sections through clearing agents and graded alcohols. Wash the sections 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, then wash with distilled water. 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. Wash the slide once with PBS. Incubate sections in the primary antibody for 20 to 30 minutes at room temperature or 37°C, then 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 avoid 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 AEC 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 the slide with tap water, distilled water, and PBS buffer, allowing the slide to remain in the 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 mounting medium.
Storage	2-8 °C
Note	For research use only
Expiration Date	12 months from date of receipt.

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