ImmunoBioScience (IBSc) Corp.

Data sheet

Immuno HRP~DAB Anti-Chicken IgY (IgG (H+L) kit

Ready-to-use for Immunohistochemistry (IHC) and Immunocytochemistry (ICC)

Catalog number: IH-8053-15 Ready to use 15 ml
IH-8053-50 Ready to use 50 ml

A. Reagents provided: 15 ml= 150 tests, and 50 ml = 500 tests, when 0.1 ml is applied per slide.

Bottle #	(IH-8053-15)	(IH -8053-50)	Description
1	15 ml	50 ml	Ready-to-use, Peroxidase Block, hydrogen peroxide (white color cap)
2	15 ml	50 ml	Ready-to-use Protein Blocking solutions (blue color cap)
3	15 ml	50 ml	Primary antibody dilution buffers (green color cap), *for Dilution of Primary antibody (this buffer does not contain Primary antibody For dilution of primary antibody, please Refer to the Data Sheet of Primary antibody*
4	15 ml	50 ml	Ready-to-use Biotinylated anti- chicken IgY(IgG (H+L) (Yellow color cap)
5	15 ml	50 ml	Ready-to-use Streptavidin conjugated to peroxidase HRP (orange color cap)
6	DAB Chromogen buffer substrate		
6B	1 ml	3 ml	Buffer concentrated (white color cap)..
6C	6Tablets	20 Tablets	DAB Chromogen Tablets (red color cap)
6S	1 ml	3 ml	Substrate concentrated (white color cap)
7	15 ml	50 ml	Ready-to-use Hematoxylin (counter stain, purple color Solution with PINK cap)
8	15 ml	50 ml	Mounting Medium (ImmunoHistoMount Red color cap)

B. Reagents required but not supplied: Washing buffer, antigen retrievers, positive or negative control and primary antibody.

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.

Intended Use: Immunohistochemistry (IHC) and Immunocytchemistry (ICC).
(This kit can be used for WB or ELISA; the dilution should be determined by the individual lab. Normally for WB the IHC reagents are diluted 2-5X and for ELISA the IHC reagents are diluted 10-100 X. For ELISA, one has to use soluble chromogen, like TMB).The optimum dilutions for WB or ELISA should be determined by the individual lab.

Storage: 2-8°C

Preparation of DAB Chromogen Reagent 6:

1. To five ml of distilled or deionized water in a test tube, add two drop of reagent B, mix well.

2. Add DAB tablet (Reagent C), let stand in this solution for 5-10 minutes. Vortex for 3-5 minutes till all DAB tablet is dissolved, add one drop of reagent S, and mix well. This ready-to-use reagent is good for several hours. *The unused DAB solution can be discarded according to city, county, state, province or country's regulations.*

Procedure: IHC/ICC procedure for frozen sections, paraffin sections and cell smears.

1. Deparafinize and hydrate tissue sections through xylene or other clearing agents and graded alcohols.(For frozen sections or cell smears; use unfixed, acetone fixed or appropriate fixative for the antigen in question; **for cell smears it may be necessary to permealize the cell by detergent, please refer to antibody protocol)**
2. Wash 2-3 with distilled or deionized water.
3. Incubate sections/cell smear in Endoblocker (#1) for 5-10 minutes at room temperature or 37°C.
   Note: If antigen retriever is required it can be applied after this stage.
4. Wash slide with PBS Tris saline **(with 0.02-0.05% nonionic detergent, Triton X100, Tween 20 or NP-40)** or washing buffer (Universal Immunoassay buffer IBSC cat # AR-6561) 3-5X.
5. Incubate sections/ cell smear in Protein blocking solution (#2) for 10 minutes. at RT or 37ºC
6. Wash slide with PBS 1X.
7. Incubate sections/cell smear in primary antibody (NOT SUPPLIED, ONLY BUFFER IS SUPPLIED FOR DILUTION) for 20-30 minutes at room temperature or 37°C. *(For more information, refer to instructions for primary antibody)*
8. Wash slide with PBS 5-7X
9. Incubate with biotinylated secondary antibody (#4) for 15 minutes at room temp. or 37°C.
10. Wash slide 5-7 times with buffer.

    Caution: Peroxidase reagents are destroyed by sodium azide and should be avoided in all buffers and regents.
11. Incubate with Streptavidin-Peroxidase reagent (5) for 10 minutes at room temperature or 37ºC.
12. Wash slide with PBS for 5-7 X.
13. Wash slide with deionized or distilled for 2-3X.
14. Incubate with DAB reagent (#6) for 5-10 minutes at room temperature or 37ºC.
15. Wash slide with distilled or deionized water 5-7X.
16. Incubate with hematoxylin counterstain (#7) 30-60 seconds.
17. Wash slide with tap water, distilled water, followed by PBS buffer.
18. Keep in this buffer for 2-3 minutes till hematoxylin change color from purple to blue.
19. Wash slide with distilled or deionized water. Now this slide is ready to be mounted with aqueous mounting medium, ImmunoHistoMount (#8) or **Organic mounting medium (not supplied)**
20. **Please see instructions for ImmunoHistoMount(The data sheet is provided)**

If organic mounting medium is used, please follow directions as described in Data sheet for the mounting medium.

<u>These are guide lines, the optimum incubation times for these reagents and reactions should be determined by the individual lab.</u>

Limitation and warranty: Our warranty is limited to the actual price paid for the product. We are not liable for any property damage, personnel injury, time, effort or economic loss due to our product.

MSDS: This product contains 0.05 % sodium azide as a preservative, appropriate care should be taken in handling. National Institute of Occupational Safety and Health has warning that sodium azide can react with lead, copper, brass or solder in the pluming system and forms hydrazoic acid in acidic condition. Discard with copious amount of water. Avoid skin and eye contact with all laboratory products. Use

appropriate lab. gear, lab coat , gloves and safety glasses. Do not ingest any lab. products. This product is not approved for administration in human or animals.

For research use only; not for use in diagnostic procedures.

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