Data sheet

Universal HRP Reagent 5 (Immuno HRP Streptavidin~peroxidase Reagent 5)

***(Ready**-to-use Reagent 5 for Immunohistochemistry (IHC) and Immunocytochemistry (ICC)*

Catalog number: IHR-8151-15 Ready to use 15 ml (Reagent 5 orange color)
IHR-8151-50 Ready to use 50 ml.(Reagent 5 Orange color)
IHR-8151- 250 (Custom F) Ready to use **250ml** (Reagent 5 orange color)

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.

Ready to use Streptavidin Peroxidase solution for IHC

Intended Use: Immunohistochemistry (IHC) and Immunocytchemistry (ICC).
(This solution an 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

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 (Immuno Automation 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 AEC reagent or DAB (#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 a aqueous mounting medium if AEC is used. With DAB either aqueous mounting medium or Organic mounting mediums can be used.

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

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.
MSD: 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.

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