

Immuno HRP AEC Anti-Chicken IGY (IgG (H+L) kit

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

Catalog number: **IH-8043-15** Ready to use 15 ml

IH-8043-50 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-8043-15)	(IH -8043-50)	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), <i>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</i>
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	AEC Chromogen buffer substrate		
6B	1 ml	3 ml	Buffer concentrated (white color cap)..
6C	1 ml	3 ml	AEC chromogen concentrated (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)

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 Immunocytochemistry (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

Mixing of Chromogen reagent 6:

1. To 5 ml of distilled or deionized water in a test tube, add two drop of reagent B buffer, mix well.
2. Add one drop of reagent C chromogen, mix well.
3. Add one drop of reagent S substrate, mix well. This ready to use reagent is good for several hours. *The unused AEC 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. Deparaffinize 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 permeabilize 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, wash with distilled water.
4. **Note: If antigen retriever (Trypsin AR-6541, Pronase AR-6542, Pepsin AR-6543, Citrate buffer AR-6544, Buffer w EDTA pH 8.5 AR-6545, Tris buffer pH 10 AR-6546) is required it can be applied at this step. Please refer to data sheet for the primary antibody.**
5. Wash slide with PBS or 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.
6. Incubate sections/ cell smear in Protein blocking solution (#2) for 10 minutes. at RT or 37°C
7. Wash slide with PBS 1X, this step can be omitted..

8. 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*)

9. Wash slide with PBS 5-7X

10. Incubate with biotinylated secondary antibody (#4) for 15 minutes at room temp. or 37°C.

11. Wash slide 5-7 times with buffer.

Caution: Peroxidase reagents are destroyed by sodium azide and should be avoided in all buffers and reagents.

12. Incubate with Streptavidin-Peroxidase reagent (5) for 10 minutes at room temperature or 37°C.

13. Wash slide with PBS for 5-7 X.

14. Wash slide with deionized or distilled for 2-3X.

15. Incubate with AEC reagent (#6) for 5-10 minutes at room temperature or 37°C.

16. Wash slide with distilled or deionized water 5-7X.

17. Incubate with hematoxylin counterstain (#7) 30-60 seconds.

18. Wash slide with tap water, distilled water, followed by PBS buffer.

19. Keep in this buffer for 2-3 minutes till hematoxylin change color from purple to blue.

20. Wash slide with distilled or deionized water. Now this slide is ready to be mounted with aqueous mounting medium, ImmunoHistoMount (#8)

21. **Please see instructions for ImmunoHistoMount(The data sheet is provided)**

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.

MSDS: Some of these products may contain 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 plumbing system and forms hydrazoic acid in acidic condition. Discard with copious amount of water. HRP reagents contain 0.05% Proclin 300. Avoid skin and eye contact with all laboratory products. Use appropriate laboratory gear, lab coats, gloves and safety glasses. Do not ingest any laboratory products. This product is not approved for administration in human or animals.

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