

Product Manual

One step Polymer HRP Mouse, Rat and Rabbit with DAB (orb2652833 / orb2652834)

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

Catalog number: orb2652833 Ready to use 50 ml

orb2652834 Ready to use 15 ml

Reagent: 15 ml= 150 tests, when 0.1 ml is applied per slide.
50 ml = 500 tests, when 0.1 ml is applied per slide.

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

Component	Volume (orb2652834)	Volume (orb2652833)
Ready-to-use, Peroxidase Block, hydrogen peroxide	15 ml Dropper bottle, tip, and white cap	50 ml Dropper bottle, tip, and white cap
Ready-to-use Protein Blocking solutions— with <i>Goat serum</i>	15 ml Dropper bottle, tip, and blue cap	50 ml Dropper bottle, tip, and blue cap
Primary antibody dilution buffers (For <i>Dilution of Primary antibody, please refer to the Data Sheet of Primary antibody. Use the buffer as negative control</i>)	30 ml Amber Bottle	80 ml Amber Bottle
One-step HRP-anti-Mouse, Rat and Rabbit IgG (H+L) Polymer Reagent	15 ml Dropper bottle, tip and red cap	50 ml Dropper bottle, tip and red cap
DAB Buffer Substrate	15 ml Natural bottle	50 ml Natural Bottle
20X DAB chromogen	1 ml Amber Vial	3 ml Amber Bottle

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, HRP anti-HRP, ABC, catalyzed signal amplification, polymer system (one or two steps) and others, to detect antigens on tissue and cells. This kit employs Polymer technology shown to provide increased sensitivity and detection. Biorbyt One-Step anti-Mouse, Rat and Rabbit IgG (H+L); biotin/avidin free system stains membranes, cytoplasmic and nuclear antigens. It provides the user with a rapid and easy to use IHC detection system.

Intended Use: Immunohistochemistry (IHC) and Immunocytochemistry (ICC).

Storage: 2-8°C, do not freeze reagents. Remove reagents from refrigerator and bring it to RT before using. After use store all reagents at 2-8°C. Positive and negative controls should be run simultaneously with the test specimens.

Preparation of DAB Chromogen Reagent 5: To one ml of buffer substrate (5BS) in a test tube, add two drop (50 µl) of reagent 5C (chromogen) mix well; this is ready-to-use DAB chromogen system. This reagent is good for 7-8 hours.

DAB solution can be discarded according to city, county, state, province or country's regulations

Procedure: IHC/ICC procedure for frozen, 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. Rinse 2-3X with distilled or deionized water.
3. Incubate paraffin sections with Endoblocker (#1) (1-3 drops to cover section) for 10 minutes at room temperature (RT). For frozen sections use Endoblocker #1 (1:10 diluted in methanol) Rinse slide with distilled water 3X.
4. Note: If antigen retriever (Trypsin orb2652916/orb2652917, Pronase orb2652914/orb2652915, Pepsin orb2652912/ orb2652913, Citrate buffer orb2652910/orb2652911, Buffer with EDTA pH 8.5 orb2652908/orb2652909, Tris buffer pH 10 orb2652906/orb2652907) is required it can be applied at this step. Please refer to data sheet for the primary antibody.

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5. Wash slide with PBS or Tris saline buffer (with 0.02-0.05% nonionic detergent, Triton X100, Tween 20 or NP-40) or washing buffer (Immuno Automation buffer Biorbyt cat # orb2652902 or orb2652903) 3X.
6. Incubate sections/ cell smear in Protein blocking solution (#2), for 10 minutes at RT. Do not rinse the slide.
7. Incubate sections/cell smear with primary antibody (NOT SUPPLIED, ONLY Primary antibody dilution BUFFER IS SUPPLIED FOR the DILUTION) as recommended by the supplier. *(For more information, refer to instructions for primary antibody).* The primary antibody dilution buffer supplied can also be used as a negative control.
8. Wash slide with PBS/buffer 5-7X
9. Incubate with One-Step HRP polymer (#4) for 15 minutes at RT.
10. Wash slide 5-7 times with PBS/buffer. *Caution: Peroxidase reagents are destroyed by sodium azide and should be avoided in all buffers and reagents.*
11. Wash slide with deionized or distilled for 2-3X.
12. Incubate with DAB chromogen reagent #5 for 5-10 minutes at RT; monitor the color development under microscope.
13. Wash slides 5-7X with distilled water.
14. Incubate with appropriate counterstain (Not supplied).
15. Wash slide with tap water, distilled water.
16. Mount slide with organic or aqueous mounting medium (not supplied). (Biorbyt aqueous mounting mediums, ImmunoHistoMount (orb2652980, orb2652981, orb2652982, orb2652983); Organic Mounting medium, Organo Mount (orb2652974, orb2652975, orb2652976, orb2652977, orb2652978, orb2652979). (Please see instructions for mounting medium.)

These are guidelines, 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.

Safety: Some of these products may contain sodium azide as a preservative, appropriate care should be taken in handling. The National Institute of Occupational Safety and Health has a 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 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 humans or animals.