DAB detection system kit (Stainer)

【REF】

LC-AS88-R001

【Package Size】

100 tests/box

【Intended Use】

DAB Detection System Kit (Stainer) is intended for laboratory use to identify immunohistochemically (IHC) marked targets in sections of formalin-fixed, paraffin embedded or frozen tissues. DAB Detection System enables visualization of stained tissues using light microscopy.

This product is intended for *in vitro* diagnostic (IVD) use.

【Principle of Procedure】

Immunohistochemistry (IHC) technique allows for the visualization of specific protein antigens in tissues for diagnostic purposes. Following the application of the primary antibody, the presence of a target antigen is visualized by the sequential application of an enzyme-labeled antibody conjugate that binds to the primary antibody, and a chromogen reagent, to produce a colored product that is visible using light microscopy. 3,3'-Diaminobenzidine (DAB) is a widely used chromogen for immunohistochemical staining process with horseradish peroxidase (HRP) detection systems.

【Specimen Collection and Preparation for Analysis】

1. Formalin-fixed, paraffin-embedded tissues.
2. Each section should be cut to the appropriate thickness of 2-5μm and placed on a positively charged glass microscope slide.

【Storage and Handling】

Store at 2-8°C. Do not freeze.

Do not use product beyond the expiration date.

【Reagents Provided】

1. Peroxidase block

Peroxidase Block contains 3-4% Hydrogen peroxide (H_2O_2).

2. Post blocking solution

Post blocking solution contains 0.4% casein in phosphate buffered saline, with stabilizers, surfactant, and 0.35% ProClin 300 as a preservative.

3. Secondary antibody

Secondary antibody contains HRP labeled antibodies (goat anti-mouse IgG, goat anti-mouse IgM and goat anti-rabbit) (<50μg/mL) in Tris-buffered saline with protein stabilizer and 0.35% ProClin 300.

4. DAB chromogen

DAB chromogen contains 1.74% w/v 3,3- diaminobenzidine, in a stabilizer solution.

5. DAB substrate buffer

DAB substrate buffer contains buffered-solution in ≤0.1% hydrogen peroxide and preservative.

6. Hematoxylin

Hematoxylin contains <0.1% hematoxylin.

【Instructions For Use】

Staining By Automatic Stainer

Refer to the appropriate Labex LC-AS88 IHC Auto Stainer Operating Manual for the staining procedure instructions.

Manual Staining

1. Deparaffinize slides in 3 changes of xylene (or Dewax solution), 15 minutes each.
2. Hydrate the slides through a graded series of alcohol dilutions, wash the section in 95%, 85% and 75% ethyl alcohol for 5 minutes each.
3. Rinse in Phosphate Buffer Saline (PBS), 3 x 2 minutes.
4. Antigen retrieval: Place slides in a pressure cooker filled with the Epitope Retrieval Solution buffer. Follow the instructions of the pressure cooker manufacturer.
5. Wash in PBS, 3 x 2 minutes.

6. Block the endogenous peroxidase by incubating the tissue in Peroxidase Block for 10 minutes.
7. Wash in PBS 3 x 2 minutes.
8. Add 100μL of primary antibody, incubate for 30 minutes at room temperature. Wash in PBS 3 x 2 minutes.
9. Add 100μL of Post Blocking Solution, Incubate for 20 minutes. Wash in PBS 3 x 2 minutes.
10. Add 100μL of secondary antibody, incubate for 20 minutes. Wash in PBS 3 x 2 minutes.
11. Prepare the DAB solution by adding 50μL of DAB Chromogen into 1mL of DAB Substrate Buffer shortly before use. Mixed carefully. Add 100μL of DAB solution onto the tissue, incubate for 3-6 minutes. Wash in PBS 3 x 2 minutes.
12. Counterstain with hematoxylin. Rinse with deionized water.

【Interpretation of Results】

1. A qualified pathologist who is experienced in IHC procedures must evaluate controls and validate the stained product before interpreting results.
2. Staining of negative controls must be evaluated first to assure that the signal generated in the staining process is not caused by nonspecific staining.

【Limitations】

1. IHC is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of the IHC slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may result from variations in fixation and embedding methods, or from inherent irregularities within the tissue.
3. The protocols for a specific application can vary. These include, but are not limited to fixation, antigen retrieval method, incubation times, and tissue section thickness.
4. The clinical interpretation of any staining, or the absence of staining, must be complemented by morphological studies and evaluation of proper controls. Results should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

【Warnings and Precautions】

1. For *in vitro* diagnostic (IVD) use.
2. Do not use product beyond the expiration date.
3. This reagent contains ProClin 300. Pregnant women and children under 18 years of age should avoid contact with the reagents. If contact happens, wash the area with copious amounts of water.

【References】

1. Clinical and Laboratory Standards Institute (CLSI) formerly NCCLS. Quality Assurance for Immunocytochemistry: Approved Guideline. CLSI document MM4-A (ISBN 1-56238-396-5). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 1999.

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