DAB Detection Kit (Manual)

【Catalog Number】

REF 6001

【Package Size】

DAB Chromogen: □1×1mL, □1×3mL

DAB Substrate Buffer: □1×1mL, □1×3mL

【Intended Use】

DAB Detection Kit (Manual) is intended to identify targets by immunohistochemistry (IHC) in sections of formalin- fixed, paraffin embedded and frozen tissue that are stained by manual and visualized by light microscopy.

【Principle of Procedure】

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

【Specimen Collection and Preparation for Analysis】

Formalin-fixed, paraffin-embedded tissues.

Each section should be cut to the appropriate thickness (2-5 μm) for the primary antibody being used 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 for the storage method.

【Reagents Provided】

1. DAB Chromogen: 1.74% w/v 3,3' - diaminobenzidine, in a stabilizer solution.
2. DAB Substrate Buffer: Buffered-solution containing ≤ 0,1% hydrogen peroxide and preservative.

【Instructions For Use】

1. Deparaffinized slides in 3 changes of xylene (or Dewax solution), 10 minutes each. and hydrate through a graded series alcohols .
2. Wash the section in 90%, 80% and 70% ethyl alcohol for 10 minutes each.
3. Rinse in distilled water, 2 x 5 minutes.
4. Block the endogenous peroxidase by incubating the tissue in 3% hydrogen peroxide (H_2O_2) for 10 minutes.
5. Wash in distilled water, 2 x 5 minutes.
6. Antigen retrieval: Place slides in a pressure cooker filled with Epitope Retrieval Solution (Citrate, pH 6.0) buffer.
7. Wash in PBS 2 x 5 minutes.
8. Add 100μL primary antibody, Incubate for 30 minutes . Wash in PBS 2 x 5 minutes.
9. Add 100μL secondary antibody (the protocol depends on the supplier), and proceed to standard immunohistochemistry protocol. Wash in PBS 2 x 5 minutes.
10. Mix 50μL DAB Chromogen and 50μL DAB Substrate Buffer in 1mL distilled water before use in the mixing vial.

    Add 100μL DAB solution, Incubate for 2-10 minutes. Wash in PBS 2min for 3 times.
11. Counterstain with hematoxylin. Rinse with deionized water.

【Interpretation of Results】

1. A qualified pathologist who is experienced in IHC procedures must evaluate controls and qualify the

stained product before interpreting results.

2. Staining of negative controls must be noted first, and these results compared to the stained material to verify that the signal generated is not the cause of nonspecific interactions.

【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, heat-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. This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

【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.
2. Elias JM, Gown AM, Nakamura RM, Wilbur DC, Herman GE, Jaffe ES, Battifora H, Brigati DJ. Special report: Quality control in immunohistochemistry. Amer J Clin Pathol 1989; 92:836-43.
3. National Committee for Clinical Laboratory Standards. Quality assurance for immunocytochemistry; approved guideline. Villanova, PA, 1999; 19(26):Order code MM4-A

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