

## Nestin

Rabbit Monoclonal Antibody

### **【Package Size】**

Ready to use:  1mL  2mL  3mL  5mL  6mL

Concentrated:  0.1mL  0.2 mL  0.5mL  1.0mL

### **【Intended Use】**

Rabbit Monoclonal anti-Nestin antibody is intended for use to qualitatively identify Nestin antigen by light microscopy in sections of formalin-fixed, paraffin-embedded tissue using IHC detection methodology.

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

### **【Principle of Procedure】**

Immunohistochemical (IHC) staining techniques allow for the visualization of antigens via the sequential application of a specific antibody to the antigen (primary antibody), a secondary antibody to the primary antibody and an enzyme complex with a chromogenic substrate with interposed washing steps. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. The specimen may then be counterstained and coverslipped. Results are interpreted using a light microscope and aid in the differential diagnosis of pathophysiological processes, which may or may not be associated with a particular antigen.

### **【Specimen Collection and Preparation for Analysis】**

Formalin-fixed, paraffin-embedded tissues.

Each section should be cut to the appropriate thickness (2-5  $\mu$ m) 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】**

**Clone: SN06-27**

Buffer: 10mM pH 7.4 Phosphate Puffer Saline (PBS).

Stabilizer: 0.05% bovine serum (BSA).

Preservative: 0.05% sodium azide (NaN<sub>3</sub>).

Ready-to-use antibody concentration: 2-5 $\mu$ g/mL.

Concentrated antibody concentration: 50-200 $\mu$ g/mL.

### **【Staining Procedure】**

#### **Staining By Automatic**

Refer to the appropriate Instrument's Operator's Manual for the complete staining procedure instructions.

#### **Staining By Manual**

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<sub>2</sub>O<sub>2</sub>) 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. Concentrated Antibody Dilution  
Suggested Dilution: 1:100-1:200  
The optimal dilution for a specific application under a given set of experimental conditions should be

determined by the investigator.

9. Add 100µL primary antibody, Incubate for 30 minutes .

Wash in PBS 2 x 5 minutes.

10. Add 100µL secondary antibody (the protocol depends on the supplier), and proceed to standard immunohistochemistry protocol. Wash in PBS 2 x 5 minutes.

11. Add 100µL DAB solution (the protocol depends on the supplier), Incubate for 2-10 minutes. Wash in PBS 2 x 5 minutes.

12. Counterstain with hematoxylin. Rinse with deionized water.

#### **【Quality Control】**

1. Differences in tissue processing and technical procedures in the user's laboratory may produce significant variability in results, necessitating regular performance of in-house controls in addition to the following procedures.
2. Controls should be fresh autopsy/biopsy/surgical specimens, formalin-fixed, processed and paraffin wax-embedded as soon as possible in the same manner as the patient sample(s).

#### **【Performance Characteristics】**

1. Positive Cellular Localization: Cytoplasmic
2. Recommended positive controls: Kidney, ovary, cervix and human melanoma tissue

#### **【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.

#### **【Warnings and Precautions】**

1. For in vitro diagnostic (IVD) use.
2. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
3. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves. If reagents come in contact with sensitive

areas, wash with copious amounts of water.

4. Avoid microbial contamination of reagents as it may cause incorrect results.
5. Consult local and/or state authorities with regard to recommended method of disposal.
- 6 Do not use product beyond the expiration date for the storage method in case of change of analytical performance of the reagent.
7. This reagent contains sodium azide. pregnancy and child under 18 age should avoid contact of reagents, If contact wash with copious amounts of water.
8. Exposure to heat, magnetic field: N/A

#### **【References】**

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2. CLSI. 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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4. Herman GE, Elfont EA. The taming of immunohistochemistry: the new era of quality control. Biotech Histochem. 1991;66(4):194-199.
5. Dabbs DJ. Diagnostic Immunohistochemistry 2010; Churchill Livingstone

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