



**N-Histofine<sup>®</sup>**

**DAB-2V**

Peroxidase Chromogen/Substrate solution

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### **3,3'-DIAMINOBENZIDINE TETRAHYDROCHLORIDE (DAB) SUBSTRATE SOLUTION SUBSTRATE FOR HORSERADISH PEROXIDASE**

Package/Code 500 tests/425312F, 1500 tests/425314F

#### **I. INTENDED USE**

For In Vitro Diagnostic Use.

**N-Histofine<sup>®</sup>** DAB-2V is designed as one of reagents to visualize antigen binding primary antibody on human tissue sections. In the immunohistochemical staining process, complex of antigen, primary antibody and enzyme-labeled secondary antibody is formed. Then the enzyme of the complex reacts with a specific chromogen/substrate solution, and accordingly colored precipitates are developed, therefore, stained antigen is observed under light microscope. This product is one of the specific chromogen/substrate solutions for peroxidase and develops brown colored precipitate by reaction with peroxidase.

For clinical diagnosis, comprehensive evaluation based on other test results and clinical symptoms as well should be undertaken besides staining results of this primary antibody, in particular, for evaluation as negative without positive result.

#### **II. COMPONENTS**

##### **1. Reagents provided**

Reagent A: DAB solution concentrate (Liquid)	...	[500 tests] 1.2 ml×2	[1500 tests] 1.2 ml×6
3,3'-diaminobenzidine tetrahydrochloride (DAB) chromogen solution			
Reagent B: DAB buffer (Liquid)	...	30 ml×2	30 ml×6
Hydrogen peroxide			

##### **2. Accessories**

Tube	...	×1	×1
Chip (For Reagent B, colored)	...	×1	×1
Dropping bottle	...	×1	×1

#### **III. USAGE**

##### **1. Preparation of DAB solution**

Add 1 drop (approx. 20 µl) of Reagent A Chromogen reagent per 500 µl of Reagent B Chromogen substrate into graduated tube, and place its cap then mix carefully without foaming.

Remove full dosage of the DAB solution from the tube to dropping bottle using pipette as well for use.

Store in light shielded condition at 2 to 8°C and use within 2 weeks after the preparation.

**Note:** 1. Use DAB solution removing foams or exclusive of foams when its surface is foamed by mixing.

2. Do not use mixture of DAB solution prepared different day.

##### **2. Addition and Reaction of DAB solution**

1. Apply DAB solution using dropping bottle covering the tissue section on the slide completely and incubate at room temperature for 5 to 20 minutes after addition, reaction and washing of peroxidase conjugate in the immunohistochemical staining process.

2. After adequate chromogenic reaction, wash the slide well with tap water.

3. Mount the slide with permanent mounting media and fix with cover slip after dehydrated in graded series of alcohol and cleared in xylene.

**Note:** 1. Adjust the reaction time by microscopic observation.

2. At the same time, staining of negative control slide using such as PBS in place of primary antibody is recommended to be prepared for checking the staining result.

### 3. Accessories

#### Tube

For mixing of Reagent A and Reagent B. (Refer to aforementioned Preparation of DAB solution.)

#### Chip (For Reagent B, colored)

Reagent B is provided with translucent chip placed on its bottle. However the chip is not allowed for applying solution drop by drop, therefore it is not suitable for small volume preparation. For such preparation, replace the translucent chip by colored chip provided exclusively for dropping is recommended. The colored chip is suitable for applying solution drop by drop.

#### Dropping bottle

Dropping bottle is used for storage of reagents, not only for dropping of DAB solution.

**Note:** Tube and dropping bottle are reusable after wash with purified water and dry completely.

### IV. STAINING PROCEDURE

Refer to the instruction\* of **N**-Histofine® Simple Stain MAX PO (MULTI) for staining procedures such as Principle, Materials required but not supplied, Specimen preparation, Control slides, Interpretation of results and General limitation. Validation is required when other detection systems besides above are used.

\*Download of the instruction is available at following website:

[http://www.nichirei.co.jp/bio/english/products/pdf/CE\\_MAXPO\\_MULTI.pdf](http://www.nichirei.co.jp/bio/english/products/pdf/CE_MAXPO_MULTI.pdf)

### V. STORAGE AND HANDLING

1. Store at 2-8°C.
2. Store prepared reagent, DAB solution, in light shielded condition at 2 to 8°C and use within 2 weeks after the preparation.
3. Confirm expiration date indicated on the labels of vial and carton before use.
4. Adjust this product to room temperature before use.
5. Refrigerate this product promptly after use.

### VI. WARNING AND PRECAUTIONS

1. For professional users.
2. Do not use this product after the expiration date.
3. Refer to MSDS of this product for further safety information.
4. Avoid inhale this product or contact with eyes, mouth skin and clothing as well.
5. Handle Reagent A with adequate attention due to its DAB evolves into mutagens is recognized.
6. Handle Reagent B with adequate attention due to hydrogen peroxide solution contained.
7. Dispose unused Reagent A and DAB solution properly in accordance with all applicable rules and regulations of local and country due to its DAB contained.

 REF	Catalog/Code Number		Temperature Limitations	 IVD	In Vitro Diagnostic Medical Device
	Manufacturer	 LOT	Batch Code		Contains Sufficient for <N> Tests
	Use By		Consult Instructions for Use	 EC REP	Authorized Representative in the European Community
	CE-mark, code of the notified body		For IVD Performance Evaluation only	 SAMPLE	Sample

**N**-Histofine is NICHIREI BIOSCIENCES's registered trademark. The registration countries would be referred us.

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