InSituVision III
IHC Detection System

Product identification

D001-15	15 ml
D001-50	50ml

Materials Provided

Label	Content	Volume (D001-15)	Volume (D001-50)
Content 1	Labelled Goat anti Mouse/Rabbit IgG polymer	15ml	50ml
Content 2A	Ultra DAB Substrate	30ml	100ml
Content 2B	Ultra DAB chromogen (20X)	1.5ml	5ml

Materials required but not provided

The following materials may be required for staining but are not provided with this product

- Microscope slides (positively charged) and cover slips
- Water bath
- Humidified chamber
- Staining jars
- Stopwatch
- Xylene or xylene substitute
- Ethanol
- Deionized or distilled water
- Primary antibody
- Positive and negative controls
- Peroxidase block
- Hematoxylin

Antigen retrieval reagent, e.g. Antigen Enhancer (HIER buffer), Cat. No. BUAN01-500
- Wash buffer: e.g. IHC Wash Buffer, Cat. No. BUWA01-1000
- Tap water/bluing reagent (e.g. ammonia water)
- Light microscope

Storage and handling

Store at 2 - 8 °C.

When stored correctly the product is stable to the expiration date indicated on the box. Do not use after expiration date.
To ensure proper reagent delivery and stability, replace the bottle caps after every use and immediately place them cool in an upright position.

Sample Preparation

Fresh biopsy or surgical samples were collected, fixed, dehydrated, and embedded into wax blocks according to the technical specifications of pathology.

The tissue was sliced and adhered to the anti-slip slide, and the tissue thickness was about 3~5μm. After removing water droplets from the tissue sections, heat them in an incubator of 60~65°C for two hours and take them out to cool to room temperature.

Staining procedure

1. Solution preparations:

DAB Visualization: Ultra DAB Substrate (Content 2A) and Ultra DAB chromogen (Content 2B) were mixed in a ratio of 20:1 (Put 50ul chromogen into 950ul substrate, for example). After preparation, immediately use it!

2. Temperature condition:

Room temperature

3. Procedures:

a）Paraffin sections were dewaxed, hydrated and rinsed with running water.
b）Epitope Retrieval
Apply antigen retrieval reagent according to instructions of primary antibody;
Rinse slides with running water for 1min; draw a circle with PAP pen outside tissue;
Rinse slides with wash buffer 2X 5min;
c）Proxidase Blocking (optional)
Remove wash buffer and drip proxidase blocking reagent on slides. Incubate it for 10min at room temperature.
Rinse slides with wash buffer, 2X 5min.
d）Adding Primary Antibody
Remove wash buffer and drip primary antibody on slides. Incubate it for 60min at room temperature.
Rinse slides with wash buffer, 2X 5min.
e）Adding Secondary Antibody
Remove wash buffer and drip Labelled Goat anti Mouse/Rabbit IgG polymer (Content 1) on slides. Incubate it for 30min at room temperature;
Rinse slides with wash buffer, 2X 5min.
f）Visualization
Remove wash buffer and drip DAB visualization (explained in solution preparation) on slides. Incubate it for 3-10 min at room temperature;
Rinse slides with wash buffer, 2X 5min.
Rinse slides with running water to stop visualization.
g）Counterstain
Remove water and drip Hematoxylin on slides. Rinse slides
with running water after 10-30 sec.
Blue slides with wash buffer and rinse with running water.
h）Dehydrate, transparent and mount
Soak in 70% ethanol for 2X 5min;
Soak in 96% ethanol, 2X 5min;
Soak in anhydrous ethanol, 2X 5min;
Soak in xylene, 2X 5min;

Mount the slide with mounting medium.
i) Observe under microscope.

4. Test Results:

Test results should be evaluated by experienced pathologist.

Interpretation of results

The immunostaining procedure causes a colored reaction product to precipitate at the antigen sites localized by the primary antibody.

A qualified pathologist experienced in immunohistochemistry procedures must evaluate positive and negative tissue controls before interpreting patient specimens.
Positive staining intensity should be assessed within the context of any background staining of the negative reagent control.

Note:

- A negative result means that the antigen in question was not detected, but not that the antigen is not present in the cells/tissues tested. An antibody panel may be used to support the results in some circumstances. Additionally, the morphology of each tissue sample should be examined utilizing a hematoxylin and eosin stained section. A qualified pathologist must interpret the patient's morphologic findings and pertinent clinical data.
- Any alterations on epitope retrieval, incubation time, temperature, or methodology could lead to unwanted results.
- Peroxidase Block should be used on tissue with abundant endogenous peroxidase content.

Warnings and precautions

1. For Research use only.
2. Application only by qualified and trained personnel and for professional persons only.
3. Take reasonable precautions when handling reagents. Use protective clothing and gloves.
4. The application of the product to non-formalin fixed tissues has not been confirmed.
5. We would not guarantee the validity of the staining result if the components in this kit are mixed with reagents from other companies.
6. Avoid microbial contamination of reagents as it may cause incorrect results.
7. Do not use reagents after expiration date.
8. There are no estimated health risks, if the product is used as directed. MSDS is available on request.
9. As with any product derived from biological sources, proper handling procedures should be used.
10. All hazardous materials should be disposed according to guidelines for hazardous waste disposal. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.

Date of publication or revision

2024-06-10
Change(s) made: -

Explanation of symbols

Catalog number

Batch code


Use by

Temperature limitation


Do not use if package damaged


Consult instructions for use


Caution