“InSituVision V” Detection System

Instructions for use

IHC Complete Detection system (Goat anti mouse/rabbit HRP, Peroxidase Quench, DAB kit)

Product identification

2 step detection system goat anti-mouse/rabbit HRP, including peroxidase quench and DAB chromogen

Product size

Cat. No.	Packing size
D002-15	15 ml
D002-50	50 ml

Intended use

For *in-vitro* application.
The kit is used for immunohistochemical detection of primary antibodies (not included in this kit) from mouse (Ms) or rabbit (Rb) in formalin-fixed, paraffin embedded (FFPE) tissue sections. The reagents included in this kit are all designed to work together.
The product can be used for manual staining and in the automatic stainer.
Detection with the antibody may only be performed by qualified personnel. The results must be evaluated by qualified pathologists taking into account the patient's medical history and other diagnostic tests.

Summary and explanation

2 Step Detection System goat anti-mouse/rabbit HRP Kits with Peroxidase Block and Chromogen (DAB) are highly sensitive and specific polymer-based detection systems. The system is used for the detection of primary antibodies derived from mouse or rabbit. The kits do not contain biotin, so the detection reaction is not affected by endogenous biotin, resulting in less background staining.

Principle of the procedure

Prior to staining, FFPE tissue sections should be processed by deparaffinization and hydration. This is followed by antigen retrieval (HIER or PIER) according to the method indicated in the package insert of the primary antibody. Endogenous peroxidase is inhibited with the included peroxidase blocking reagent. Blocking of nonspecific binding with a protein-blocking reagent is an optional step in the procedure. The use of ready to use primary antibodies is recommended. If antibody concentrates are used, the optimal dilution must be determined in your own system. Polymer-based methods are used for signal amplification. The secondary antibody is conjugated to the enzyme HRP using a synthetic polymer support. This approach increases the number of available enzymes or ligands, resulting in a higher turnover rate of the chromogen. Enzymatic activation of the chromogen results in a visible reaction product at the antigen site. Application of the attached and freshly prepared DAB reagent results in a brown product. Each step is incubated for a precise time and requires intermediate wash steps with recommended wash buffer. The final step is counterstaining, which yields a blue nuclear stain. This is followed by mounting with a mounting medium. For information on product recommendations, please refer to the section "Additional material required but not supplied". The results are interpreted under the light microscope.

Materials provided

Tube Label	Description
Reagent 1 (R1)	Peroxidase Blocking Reagent (Peroxidase Quench)
Reagent 2 (R2)	- Primary antibody enhancer
Reagent 3 (R3)	Anti-mouse/rabbit polymer-HRP
Reagent 4 (R4)	Substrate Buffer
Reagent 5 (R5)	DAB Chromogen

The batch number is indicated on the product label.

Both reagents are ready to use and have been optimized for staining. No further dilution, reconstitution, mixing or titration is required.

Materials required but not provided

The following materials may be required for staining with the polymer system, but are not supplied.
- Positive and negative controls
- Slides (positively charged) and coverslips
- Water bath with accurate temperature recording
- humidity chamber
- Staining jars
- Stopwatch
- Xylene or xylene substitute
- Ethanol
- Deionized or distilled water
- Primary antibody, ready to use or concentrate
- Antibody diluent solution
- Antigen retrieval reagent, e.g. Antigen Enhancer (HIER buffer), Cat. No. D004
- Protein Blocking Reagent
- Hematoxylin
- Mounting medium
- Wash buffer, e.g. IHC Wash Buffer, Cat. No. D003
- 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 until the expiration date printed on the vial. Do not use the reagent after the expiration date.

To maintain proper reagent delivery and product stability, replace the cap after each use and immediately refrigerate the vial in an upright position.

Warnings and precautions

1. Use only by trained and qualified personnel.
2. If used as directed, the product is not classified as a hazardous substance, so that no health hazard is to be expected. The safety data sheet is available on request.
3. As with all products of biological origin, these must be handled appropriately.
4. Do not use the reagents after the expiry date.
5. Take appropriate precautions when handling reagents. Wear appropriate protective clothing when working.
6. Manage waste disposal in accordance with local, state, and federal standards. Materials of human or animal origin must be handled as biohazardous materials and disposed of with proper precautions.
7. Avoid microbial contamination of reagents, as this may cause erroneous results.

Specimen preparation

The kit is suitable for FFPE tissue sections. The staining conditions are based on specimens with a section thickness of 2 - 5 µm. The sections must be fixed and well prepared. 10% neutral-buffered formalin is recommended as fixative. Different results may occur as a result of prolonged tissue fixation or special processes such as decalcification of bone tissue.
Preparations should be stained as soon as possible as antigenicity decreases with time.
It is recommended that positive and negative controls always be stained along with the unknown specimens.
The best possible procedures are determined and verified by the user.

Reagent preparation

All reagents are ready to use and only need to be brought to room temperature (RT) before starting.
Only DAB chromogen has to be prepared freshly before use.
To 1 ml substrate buffer (R4) add one drop (50 µl) of DAB Chromogen (R5) and mix well. The solution is now ready for use and should be used within 1 to 2 hours.

Staining procedure

The following data are recommendations. Due to differences in tissue fixation and processing, as well as general characteristics of the laboratory equipment used and prevailing laboratory conditions, it may be necessary to adjust incubation times. The best possible procedure will be determined and verified by the user.
If using an autostainer, carefully read the instruction manual before use. The recommended reagent volume is 200 µl per slide.

1. Deparaffinization and rehydration of the tissue sections on the slides.
2. Wash in buffer (TBS or PBS) for 2 x 5 min.
3. Enzyme digestion or heat pretreatment can be performed if required (see primary antibody data sheet). A suggested HIER protocol: Preheat Antigen Enhancer (HIER buffer) to 95 - 99 °C and incubate sections in it for 20 min. Cool sections in hot Antigen Enhancer to RT for 20 min. Wash sections in IHC wash buffer for 2 x 5 min, drain off liquid.
4. Blocking of endogenous peroxidase with the included peroxidase blocking reagent (R1) 5 min
Apply enough volume to completely cover the tissue section.
5. Wash in IHC wash buffer 2 x 5 min
6. Primary Antibody 60 min
Apply enough volume to completely cover the tissue section. Incubate in humidity chamber at RT. Apply sufficient volume to completely cover the tissue section.
7. Wash in IHC wash buffer 2 x 5 min
8. Apply Primary antibody Enhancer (R2). 15 min Apply enough volume to completely cover the tissue section.
9. Wash in IHC wash buffer 2 x 5 min
10. Apply Polymer Ms/Rb HRP (R3) 30 min
Apply enough volume to completely cover the tissue section.
11. Wash in IHC wash buffer 2 x 5 min
12. Chromogen: DAB 5 - 10 min
Prepare and incubate the solution according to the working instructions of the chromogen supplied (R4, R5). Apply enough volume to completely cover the tissue section.
13. Wash with Aqua Dest. 2 x 2 min
14. Manual counterstaining with hematoxylin 1 min
15. Rinse well with Aqua Dest.
16. Mounting with suitable mounting media.
17. Evaluation of the sections under the light microscope.

Quality control procedures

Positive Tissue Control
A positive tissue control must be included with each staining run to verify proper performance of the processed tissue and reagents. If adequate positive staining cannot be provided with the positive tissue control, the results must be considered invalid with the patient samples.

Negative Tissue Control
Negative tissue controls should indicate nonspecific staining. If specific staining is present in the negative control, the results must be considered invalid with the patient specimens.

The variety of cell types found in most tissues provides sites for internal negative control, so the same tissue can be used for the negative tissue control as for the positive tissue control.

Discrepancies
If quality control results do not meet specifications, patient results are invalid. The problem must be identified and corrected. The entire procedure can then be repeated with the patient samples.

Negative control reagent
For each sample, a negative control reagent is carried in place of the primary antibody to evaluate nonspecific staining. Host species and incubation time of the negative control reagent should be according to the primary antibody.

Interpretation of results

At the end of the procedure, there is a colored reaction product at the antigen site localized by the primary antibody.

DAB results in brown reaction products.
Chromogen-specific color should appear in the positive control at the expected sites. Nonspecific staining can be recognized by the fact that it appears as rather diffuse on the slides treated with the negative control reagent. The nuclei are stained blue by the hematoxylin counterstain.

Positive and negative controls are initially evaluated by a qualified pathologist. If the control slides are suitable, evaluation of the patient samples can begin. The intensity of the positive staining must be evaluated in light of the 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. In addition, the morphology of all tissue samples should be examined using a hematoxylin/eosin-stained section. Interpretation of the morphologic findings of the patient specimens as well as the clinical data should only be performed by a qualified pathologist.

Limitations

1. This reagent is “for professional use only” as immunohistochemistry is a multiple step process that requires specialized training in the selection of the appropriate reagents, tissues, fixation and processing, preparation of the immunohistochemistry slide, choice of detection system, and interpretation of the staining results.
2. Tissue staining is dependent on the handling, processing and storage 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 incorrect results. Optimal performance requires adequate specimen quality as well as appropriate sample preparation.
3. Excessive or incomplete counterstaining may compromise proper interpretation of results.
4. False positive results may be seen because of nonimmunological binding of proteins or substrate reaction products. They may also be caused by pseudo peroxidase activity (erythrocytes), endogenous biotin (example: liver, brain, kidney) or endogenous peroxidase activity (cytochrome C).
5. When used in blocking steps, normal sera from the same animal source as the secondary antisera may cause false negative or false positive results because of the effect of autoantibodies or natural antibodies.
6. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen may exhibit nonspecific staining with HRP.
7. Unexpected results may occur due to biological variability of antigen expression in neoplasms or other pathological tissues.
8. The clinical interpretation of any test results should be evaluated within the context of the patient’s medical history and other diagnostic laboratory test results. Staining must be performed in a certified, licensed laboratory under the supervision of a qualified pathologist who is responsible for evaluation and assuring the adequacy of positive and negative controls. Manufacturer is not liable for incorrect results due to visual evaluation.
9. Prediluted antibodies are ready-to-use and optimized for staining. Further dilution may lead to incorrect results.
10. The performance of the product was established using the procedures provided in this package insert only and modifications to these procedures may lead to changes in efficiency. Non-application as prescribed in this data sheet leads to loss of all liability. Any changes in product, composition, implementation, as well as use in combination with any reagents other than recommended herein is not allowed; users are responsible themselves for those changes and have to perform prior validation.
11. Application in combination with diagnostic devices requires prior validation.

Troubleshooting

1. Only intact cells should be used for interpretation of staining results, as degenerated cells show nonspecific staining.
2. If no staining occurs, control application order of reagents. Follow all indications given in the instructions for use.
3. Do not allow the sections to dry out.
4. If weak staining occurs, pay attention during staining steps to freshly prepared chromogen, incubation times and temperatures, as well as accurate draining off of reagents.
5. Avoid surplus background staining by optimal removal of paraffin, washing of slides and dilution of

primary antibody. If excessive background staining occurs, high levels of endogenous biotin may be present (unless a biotin-free detection system is being used). A biotin blocking step should be included.

6. Sodium azide inactivates HRP, which may lead to false results. Wash sections in sodium azide free buffer.
7. Contact customer service in case of any uncertainties.

Date of publication or revision

2022-05-08
Change(s) made: -

Explanation of symbols

Catalog number

Batch code


Use by

Temperature limitation


Do not use if package damaged