intelliPATH™ Universal HRP Detection Kit

Mouse or Rabbit Primary Antibodies
Detection Kit
Control Number: 901-IPK5011-090617

Catalog Number:	Description:
IPK 5011 G80	80 ml kit

Intended Use:

For In Vitro Diagnostic Use
intelliPATH™ Universal HRP Detection is intended for laboratory use in the qualitative detection of mouse IgG, mouse IgM, and rabbit primary antibodies in immunohistochemistry (IHC) procedures on formalin-fixed paraffin-embedded (FFPE) tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary & Explanation:

Biocare's Universal HRP Detection Kit has been developed for use with mouse and rabbit primary antibodies. This biotin-free technology uses a specific probe to detect mouse primary antibodies, which is then followed by a horseradish peroxidase (HRP) polymer that binds to the probe or to rabbit antibodies.

intelliPATH Peroxidase Blocking Reagent is a stable form of hydrogen peroxide for blocking endogenous peroxidase.

3, 3' Diaminobenzidine (DAB) is a widely used chromogen for immunohistochemical staining. In the presence of peroxidase enzyme, DAB produces a brown precipitate that is insoluble in alcohol and xylene. This product comes in a two-component system consisting of a liquid stable DAB chromogen and DAB substrate buffer.

intelliPATH Hematoxylin is water-based and is specially formulated for immunohistochemistry. intelliPATH Hematoxylin stains nuclei a beautiful sky-blue and provides high contrast staining for DAB procedures.

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As:

80ml

intelliPATH™ Mouse Secondary Reagent (IPSC5001G20) 20ml x 4
intelliPATH™ Universal HRP Tertiary Reagent (IPT5002G20) 20ml x 4
intelliPATH™ DAB Chromogen (IPC5008G3) 3ml x 4
intelliPATH™ DAB Buffer (IPBF5009G20) 20ml x 8
intelliPATH™ Peroxidase Blocking Reagent (IPB5000G20) 20ml x 4
intelliPATH™ Hematoxylin (IPCS5006G20) 20ml x 4

Materials and Reagents Needed But Not Provided:

Microscope barrier slides, positively charged
Desert Chamber* (Drying oven)
Positive and negative tissue controls
Xylene (Could be substituted with xylene substitute*)
Ethanol or reagent alcohol
Decloaking Chamber* (Pressure cooker)
Deionized or distilled water
Wash buffer*
Pretreatment reagents*
Enzyme digestion*
Primary antibody*
Negative control reagents*
Mounting medium*

* Biocare Medical Products: Refer to a Biocare Medical catalog for further information regarding catalog numbers and ordering information. Certain reagents listed above are based on specific application and detection system used.

Species Reactivity:

Mouse and Rabbit IgG heavy and light chains.

Storage and Stability:

Store at 2ºC to 8ºC. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Protocol Recommendations:

Deparaffinization: Deparaffinize slides in Slide Brite or xylene. Hydrate slides in a series of graded alcohols to water.
Pretreatment Solution/Protocol: Please refer to the respective primary antibody data sheet for recommended pretreatment solution and protocol.
Peroxide Block: Block for 5 minutes with intelliPATH Peroxidase Blocking Reagent.
Primary Antibody: Please refer to the respective primary antibody data sheet for incubation time.
Secondary Reagent: (Mouse antibodies only) Incubate for 10-20 minutes for mouse antibodies at room temperature (RT).
Tertiary Reagent: Incubate for 10-20 minutes for mouse antibodies or 30 minutes for rabbit antibodies at RT .
Chromogen: Incubate for 5 minutes at RT with intelliPATH DAB.
Counterstain: Rinse with deionized water. Incubate for 5 minutes with intelliPATH Hematoxylin. Rinse with TBS Buffer for 1 minute followed by a rinse with deionized water.

Technical Notes:

1. Primary antibody titers can be dramatically increased when using Biocare's Revival Series Diluents and Heat Retrieval Solutions.
2. Do not use goat serum as a protein block.

Quality Statement:

Biocare protocols have been standardized using in-house antibodies, detection and accessory reagents for use on the intelliPATH automated stainer. Recommended staining protocols are specified in the data sheet of the antibody of interest. Pre-optimized intelliPATH protocols with preset parameters can be displayed, printed and edited according to the procedure in the Operator's Manual. Refer to the Operator's Manual for additional instruction to navigate intelliPATH software and stainer. Use TBS for washing steps unless otherwise specified.

Limitations:

The protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2). CLSI Wayne, PA, USA (www.clsi.org). 2011

Precautions:

1. This product is not classified as hazardous. The preservative used in this reagent is Proclin 950 and the concentration is less than 0.25%. Overexposure to Proclin 950 can cause skin and eye irritation and irritation to mucous membranes and upper respiratory tract. The concentration of Proclin 950 in this product does not meet the OSHA criteria for a hazardous substance. Wear disposable gloves when handling reagents.

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Precautions Cont'd:

2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water.
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The MSDS is available upon request and is located at http://biocare.net/support/msds/.
7. Consult OSHA, federal, state or local regulations for disposal of any toxic substances.

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Warnings:

1. DAB is known to be a suspected carcinogen.
2. Do not expose DAB components to strong light or direct sunlight.
3. Wear appropriate personal protective equipment and clothing.
4. DAB may cause sensitization of skin. Avoid contact with skin and eyes.
5. Observe all federal, state and local environmental regulations.

Troubleshooting:

Follow the reagent specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

Troubleshooting Guide:

No Staining

1. Critical reagent (such as primary antibody) omitted.
2. Staining steps performed incorrectly or in the wrong order.
3. Heat-induced epitope retrieval (HIER) step was performed incorrectly using the wrong time, the wrong order or the wrong pretreatment.
4. Insufficient amount of antigen.
5. Secondary antibody at too low of a concentration.
6. Primary antibody incubation period too short.
7. Improperly mixed substrate and/or chromogen solution(s).

Weak Staining

1. Tissue is either over-fixed or under-fixed.
2. Primary antibody incubation too short.
3. Low expression of antigen.
4. Heat-induced epitope retrieval (HIER) steps performed incorrectly using wrong time, in the wrong order, or the wrong pretreatment.
5. Over-development of substrate.
6. Excessive rinsing during wash steps.
7. Omission of critical reagent.
8. Incorrect procedure in reagent preparation.
9. Improper procedure in test steps.

Non-specific or High Background Staining

1. Tissue is either over-fixed or under-fixed.
2. Incorrect blocking reagent used; blocker should be from same species in which the secondary antibody was raised.
3. Tissue may need a longer or a more specific protein block.
4. Substrate is overly-developed.
5. Tissue was inadequately rinsed.
6. Deparaffinization incomplete.
7. Tissue damaged or necrotic.

Troubleshooting Cont'd:

Tissues Falling off

1. Slides were not positively charged.
2. A slide adhesive was used in the waterbath.
3. Tissue was not dried properly.
4. Tissue contained too much fat or poorly processed.

Specific Staining Too Dark

1. Concentrated antibody not diluted out properly (being used at too high of a concentration).
2. Incubation of primary antibody, or detection too long.

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