Diagnostic BioSystems

IHC Made Affordable

Mohs HRP-Green KIT

Catalog Number: K092

Document #: DS-2059-A

Effective Date: 07/28/2020

Intended Use

For In Vitro Diagnostic Use.

Summary and Explanation

The Diagnostic BioSystems HRP-Green kit is designed for rapid IHC staining of frozen tissue sections and paraffin-embedded tissue sections. When the time to final results is critical, such as Mohs micrographic surgery, this Mohs HRP-Green Kit can provide reliable IHC results in less than 20 minutes. Specifically for frozen sections. This Mohs HRP-Green kit utilizes a permeabilization pretreatment step in a microwave oven, without boiling, This step has been shown to increase staining intensity compared with no permeabilization pretreatment. 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 and proper controls interpreted by a qualified pathologist and/or physician.

Features

- Improved accessability of antigens in frozen tissue sections
- Green chromogen provides better contrast to endogenous melanin.
- Rapid two-step immunohistochemistry protocol
- Time to results in < 20 minutes
- No rinse step required after primary antibody
- Optimized for use with Diagnostic BioSystems Ready to use IHC antibodies.

Known Applications

Rapid Immunohistochemistry
Mohs Micrographic Surgery
Frozen Tissue Sections
Paraffin-embedded Tissue Sections

Product Description

The Diagnostics BioSystems Mohs HRP-Green Kit consists of a ready-to-use HRP-Polymer, a concentrated solution of PermaGreen HRP chromogen, and a PermaGreen HRP Substrate Buffer for dilution of the HRP Green Chromogen. This Kit is optimized for detection of mouse primary antibodies. After an initial incubation of the tissue with the selected primary antibody, the HRP-Polymer is next applied to the tissue section. The working HRP Green solution is prepared by diluting the HRP Green Chromogen with the HRP Green Substrate Buffer. After incubation with the HRP-Polymer the tissue is next incubated with the working solution of HRP Green. The development of a green reaction product indicates the presence of the antigen of interest, whereas the absence of a green reaction product indicates the absence of the antigen of interest.

Format

Cat #	Name	Format	Volume
PC001	HRP-Polymer	Ready-To-Use	20ml
K074B	PermaGreen/HRP Substrate buffer	Ready-To-Use	30ml
K074C	PermaGreen/HRP Chromogen	50X Concentrate	1ml
K095	Purple Hematoxylin	Ready-To-Use	20ml
K043	10X Tris EDTA Buffer For Permeabilization pretreatment pH 9.0	10X Concentrate	50ml
K093	Rapid Histo-Sealer (10X)™	10X Concentrate	10ml

Principles of the Procedure

Frozen Tissue sections may be fixed by air drying. If formalin is used as a fixative, such as with paraffin-embedded formalin fixed tissues, heat induced antigen retrieval (HIER) may be required.

Other methods of tissue fixation may require validation by user to ensure that antigens of interest are not harmed by the method of fixation.

Tissue sections are incubated with a primary antibody that will bind to its corresponding antigen, if present. Next, the HRP-Polymer is applied to the tissue section and binds to the primary antibody. The antibody-HRP complex next catalyzes the oxidation of HRP-Green to form a visible green reaction product at the site of the antigen-antibody reaction. The tissue may then be further processed by counterstaining and cover slipping. Results are viewed and interpreted using a light microscope.

Materials Required But Not Provided

Diagnostic BioSystems Primary Antibodies for Mohs: See Table Below
Rinse Buffer Cat. #K005
Permanent Mounting Medium
Coverslips

Storage and Handling

Store at 4°C. Do not use after expiration date printed on label. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Working solutions of HRP Green Substrate should be used promptly.

Specimen Preparation

Frozen Tissue Sections:

Frozen Tissue Sections should be cut at about 4-6 microns and affixed to microscope slides that have been treated for tissue adherence, such as positively charge microscope slides. The frozen tissue section may be air-dried onto the slide. The frozen tissue section should be stained as soon as possible to prevent antigen deterioration. Slides may be stored at 4 C for up to 48 hours.

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Precautions

1. Wear disposable gloves when handling reagents.
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 label.
6. The MSDS is available upon request.
7. Consult OSHA, federal, state or local regulations for disposal of any toxic substances.

Preparation of Working Solutions

PermaGreen/HRP

1. The 50X concentrated PermaGreen/HRP Chromogen should be diluted with the PermaGreen/HRP Substrate buffer.
2. Mix one drop (~20µl) of concentrated PermaGreen/HRP Chromogen into 1.0 mL of PermaGreen/HRP Substrate buffer.
3. Shake the bottle vigorously to completely mix the components.
4. The working solution is stable up to 4 hours after preparation. For optimal results prepare fresh reagent.

Tris EDTA Buffer

Mix 1 part of 10X Tris EDTA with 9 parts of distilled water

Rapid Histo-Sealer ™Solution:

Prepare Working Rapid Histo-Sealer™solution by diluting the 10X Rapid Histo-Sealer™reagent with Reagent Alcohol in a ratio of 1 part 10X Rapid Histo-Sealer™ to 9 parts reagent alcohol

Protocol Recommendations (Frozen Section)

1. Pre-heat Permeabilization solution in the microwave for 1 minute (Microwave setting = 50%).
2. Place microscope slide with adherent tissue section into the Permeabilization buffer and microwave slides for 2 minutes (microwave setting = 30%) . Do not allow solution to boil.
3. Remove slide and wipe around tissue section to remove excess buffer.
4. Apply primary antibody to tissue section and incubate for five minutes.
5. Without rinsing drain off excess primary antibody, and then apply HRP-Polymer for five minutes.
6. Rinse thoroughly with rinse buffer to remove excess unbound HRP-Polymer.
7. Wipe around tissue section to remove excess buffer.
8. Apply working solution of HRP-Green Substrate, and incubate for five minutes.
9. Rinse off excess HRP-Green Substrate with deionized or distilled water.

Counterstain

The tissue counterstained with Purple Hematoxylin.

1. Rinse slides with deionized or distilled water.
2. Place slides into Purple Hematoxylin for 30-60 seconds.
3. Rinse slides in deionized or distilled water until all free hematoxylin has been removed.
4. Bluing the slides in alkaline solution is not required and is not recommended.

Rapid Histo-Sealer™ and Mounting

The following procedure is designed for rapid drying and mounting of slides.

1. Prepare Working Rapid Histo-Sealer™solution by diluting the 10X Rapid Histo-Sealer™reagent with Reagent Alcohol in a ratio of 1 part 10X Rapid Histo-Sealer™ to 9 parts reagent alcohol.
2. For single slide processing, wipe excess water from both sides of the slide.
3. While holding the slide vertically, slowly pipette the working Rapid Histo-Sealer™ Dry solution down the face of the slide allowing the solution to remove any remaining water on the slide. Approximately 0.5 mL of working Rapid Histo-Sealer™ solution will be sufficient to completely remove the water from the slide.
4. After the water has been completely removed, wipe any excess Rapid Histo-Sealer™ reagent from the slide.
5. Lay the slide horizontally and allow the slide to completely dry. Drying time is approximately 10 -20 seconds. The Rapid Histo-Sealer™ reagent contains polymers that will prevent the creation of drying artifacts upon drying.
6. Once the slides have completely dried, they can be mounted with any resin-based Permanent mounting medium.
7. Apply a glass coverslip.

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

Troubleshooting

Contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2, techsupport@dbiosys.com or your local distributor to report unusual staining.

Limitations of the Procedure

Interpretation of the staining results is solely the responsibility of the user.

Warranty

There are no warranties, expressed or implied, which extend beyond this description. Diagnostic BioSystems is not liable for property damage, personal injury, or economic loss caused by this product.

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Expected Results

If the antigen of interest is present, a green-blue reaction product will be deposited at the site of the antigen. If the antigen of interest is absent, no green-blue reaction product will be deposited.

First view the negative control and verify that there is no green staining present. Next view the patient sample. If the negative control shows no staining, then a green-blue stain in the patient sample is indicative of a positive test.

Performance Characteristics

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 Diagnostic BioSystems products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist

References

I. Sroa N, *et al*. Immunohistochemistry in Mohs Micrographic Surgery. A Review of the Literature. J Clin Aesthet Dermatol 2: 37-42, 2009.

II. Stranahan D, *et al.* Immunohistochemical stains in Mohs surgery: A review. Dermatol Surg 35: 1023-1034, 2009.

III. Trimble JS, *et al*. Rapid Immunostaining in Mohs: Current Applications and Attitudes. Dermatol Surg 39: 56-63, 2013.

Mohs Pre-diluted Abs Optimized for Kit K092

Antibody	Clone	Volume	New Pre-dilute Catalog #
Pan Cytokeratin	AE1/AE3	6ml	PDM072
Epithelial Antigen	Ber-EP4	6ml	PDM131
CD34	QBEND/10	6ml	PDM050
Cytokeratin	CAM 5.2	6ml	PDM181
Cytokeratin 7	LP1K	6ml	PDM563
MART-1	A103	6ml	PDM153
Melanoma	HMB45	6ml	PDM011
S100	4C4.9	6ml	PDM194
MiTF	D5	6ml	PDM168
SOX-10	20B7	6ml	PDM565

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