

Green Chromogen

Catalog No: DC9913

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Instructions for Use

Please read before using this product.

Check the integrity of the packaging before use.

Green Chromogen

Catalog No: DC9913

Intended Use

This detection component is for *research use only*. Not for use in diagnostic procedures.

Green Chromogen is to be used in conjunction with BOND Polymer Refine HRP PLEX Detection (Catalog No. DS9914) for the detection of tissue-bound mouse and rabbit IgG as well as some mouse IgM primary antibodies. It is intended for staining sections of formalin-fixed, paraffin-embedded tissue on the BOND RX and BOND RXm automated system.

Green Chromogen must be used with laboratory best practice in the use of tissue controls. For assurance, laboratories should stain each sample in conjunction with positive, negative, and other tissue specific controls as needed.

Summary and Explanation

Immunohistochemical techniques can be used to demonstrate the presence of antigens in tissue and cells (see "Using BOND Reagents" in your BOND user documentation).

Slides generated using Green Chromogen can be dehydrated and cleared via conventional procedures with alcohol and xylene. Slides generated using Green Chromogen are recommended to be counterstained with Hematoxylin Counterstain (Catalog No. AR9915). Please refer to Product Specific Limitations section for further information.

For multiplex information please refer to the multiplex guidance section.

Using Green Chromogen in combination with the BOND automated system reduces the possibility of human error and inherent variability resulting from individual reagent dilution, manual pipetting and reagent application.

Reagents Provided

Reagents provided are sufficient for 20 individual BOND staining runs, a maximum of 100 slides.

To achieve a maximum of 100 slides from this Green Chromogen, slides must be batched in quantities of 5 per Slide Staining Assembly. Batching in quantities of less than 5 will result in fewer stained slides.

- Green Part A (15 mL)
- Green Part B (15 mL)

Dilution and Mixing

Green Chromogen is optimized for use on the BOND RX and BOND RXm system. Reconstitution, mixing, dilution, or titration of these reagents is not required.

Materials Required But Not Provided

- BOND Polymer Refine HRP PLEX Detection (Catalog No. DS9914) is required to obtain IHC and ISH results.
- Hematoxylin Counterstain (Catalog No. AR9915).

Refer to "Using BOND Reagents" in your BOND user documentation for a complete list of materials required for specimen treatment and immunohistochemical staining using the BOND system.

Storage and Stability

Store at 2–8 °C. Do not freeze. Do not use after the expiration date indicated on the label. Return to 2–8 °C immediately after use.

There are no obvious signs to indicate instability of this product, therefore positive and negative controls should be run simultaneously with unknown specimens (refer to "Quality Control" in the "Using BOND Reagents" section of your BOND user documentation).

If unexpected staining is observed that cannot be explained by variations in laboratory procedures and a problem with the Green Chromogen is suspected, contact your local distributor or the regional office of Leica Biosystems immediately.

Storage conditions other than those specified above must be verified by the user¹.

Precautions

- This detection component is for research use only. Not for use in diagnostic procedures.
- To obtain a copy of the Material Safety Data Sheet contact your local distributor or regional office of Leica Biosystems, or alternatively, visit the Leica Biosystems' website, www.LeicaBiosystems.com.
- 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 precautions². Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents or specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. Seek medical advice.
- Consult Federal, State or local regulations for disposal of any potentially toxic components.
- Measures to minimize microbial contamination of reagents need to be taken to prevent the occurrence of non-specific staining.

Instructions for Use

Scan each of the barcode labels on Green Part A and Green Part B containers to register the reagents into the BOND Reagent Inventory. Load both containers into a BOND Reagent Tray and load onto the BOND RX or BOND RXm.

Green Chromogen was developed for use on the BOND RX and BOND RXm automated system using *IHC Protocol T and *ISH Protocol W.

*IHC Protocol T and *ISH Protocol W includes BOND Polymer Refine HRP PLEX Detection (Catalog No. DS9914) and Hematoxylin Counterstain (Catalog No. AR9915) dispense and incubation steps. All three products are required to run *IHC Protocol T and *ISH Protocol W.

Operating parameters for application of the detection system reagents on the BOND Processing Module have been optimized at Leica Biosystems. These can be displayed by following the instructions in your BOND user documentation.

Product Specific Limitations

Green Chromogen has been optimized at Leica Biosystems for use with BOND Polymer Refine HRP PLEX Detection (Catalog No. DS9914), Hematoxylin Counterstain (Catalog No. AR9915) and BOND ancillary reagents. Laboratories may use their own primary antibodies provided they have been diluted to an appropriate concentration with BOND Primary Antibody Diluent (Catalog No. AR9352). Users who deviate from recommended test procedures must accept responsibility for interpretation of results under these circumstances.

The appropriate concentration of user's own primary antibodies may vary, due to variation in tissue fixation and the effectiveness of antigen enhancement and must be determined empirically. Negative reagent controls should be used when optimizing retrieval conditions and primary antibody concentrations.

If the contrast between Green Chromogen specific staining and Hematoxylin Counterstain (Catalog No. AR9915) is deemed insufficient by the user a recommended dilution of 1/3 of Hematoxylin Counterstain (Catalog No. AR9915) with Deionised water can be used in place of Hematoxylin Counterstain.

Green Chromogen must be used with laboratory best practice in the use of tissue controls. For assurance, laboratories should stain each sample in conjunction with positive, negative and other tissue specific controls as needed.

Multiplex Guidance

Green Chromogen (Catalog No. DC9913) can be used in conjunction with Blue Chromogen (Catalog No. DC9896), BOND Polymer Refine Red Detection (Catalog No. DS9390) and BOND Polymer Refine Detection (Catalog No. DS9800) to generate multiplex slides.

The order of detection can be optimised by the user however, the Green Chromogen must be the last layer of detection otherwise weak or insufficient staining can be obtained.

When multiplexing please take into consideration the compatible dehydration and mounting procedures of all chromogens included.

Troubleshooting

Refer to reference 3 for remedial action.

If the result does not correspond to the expected results with the use of controls, the test should be repeated.

If the staining result is not as expected and you wish to troubleshoot performance of the instrument and detection system independently, your local Leica representative can provide specific protocols. The ancillary reagent must be used in conformance to the package instructions and within the shelf life indicated on the product itself.

Further Information

Further information on immunostaining with BOND reagents, under the headings Principle of the Procedure, Materials Required, Specimen Preparation, Quality Control, Assay Verification, Interpretation of Staining, Key to Symbols on Labels, and General Limitations can be found in "Using BOND Reagents" in your BOND user documentation.

Bibliography

1. Clinical Laboratory Improvement Amendments of 1988, Final Rule 57 FR 7163 February 28, 1992.
2. Villanova PA. National Committee for Clinical Laboratory Standards (NCCLS). Protection of laboratory workers from infectious diseases transmitted by blood and tissue; proposed guideline. 1991; 7(9). Order code M29-P.
3. Bancroft JD and Stevens A. Theory and Practice of Histological Techniques. 4th Edition. Churchill Livingstone, New York. 1996.

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