



ihc Magenta 1:1 Kit

K50011-030, 300 tissue stains*

K50011-015, 150 tissue stains*

Summary and Explanation:

The Novodiox ihc Magenta 1:1 Kit reagents form a magenta (or reddish-purple) colored chromogen dye and is intended for laboratory use for immunohistochemistry (IHC), immunocytochemistry (ICC) or other immunoassays. The ihc Magenta 1:1 Working Solution (WS) should be prepared prior to performing an assay by mixing equal volumes of the Magenta Chromogen and the Magenta Diluent. This WS will then serve as a substrate for immunoassays that utilize horseradish peroxidase (HRP) or polymerized HRP (pHRP) enzymes.

Principle of Procedure:

The ready-to-use Novodiox immunoassays pHRP conjugated antibodies are applied to formalin-fixed paraffin-embedded (FFPE) pretreated, fresh, or frozen tissue sections, where they bind to the matching antigen. After a thorough washing step, the ihc Magenta 1:1 WS is applied to the same tissue. The antibody-linked pHRP catalyzes the chromogen substrate to form visible magenta-colored precipitate at the site of the antibody-antigen reaction. The specimen may then be further processed or counterstained with hematoxylin and a coverslip applied. Results are viewed and interpreted using a light microscope.

Reagents Provided:

K50011-### Kit Components	∇	Component Part Numbers	Items /Kit	Sizes
Magenta Chromogen	300*	C30020-G015ML	1	7.5ml
	150*	C30020-G7.5ML	1	15ml
Magenta Diluent	300*	C30021-G015ML	1	7.5 ml
	150*	C30021-G7.5ML	1	15 ml

* At estimated volume of 100µl ihc Magenta WS per tissue

Storage and Handling:

The ihc Magenta 1:1 Kit should be stored at 2-8°C. Do not freeze. The ihc Magenta 1:1 WS should be made fresh or used within the stated WS shelf life prior to use. The WS is stable once prepared and stored at 2-8°C for 7 days from when the reagents were mixed. This kit is suitable for use until expiry date when stored at 2-8°C. Do not use the product after expiration date. If reagents are stored under any conditions other than those specified in the package insert, they must be verified by the user.

Preparation and Instructions for Use for ihc Magenta 1:1 Working Solution:

1. Read and understand antibody test instructions prior to using ihc Magenta 1:1.
2. To prepare the ihc Magenta 1:1 WS, dispense equal drops or parts (e.g. 200µl) of the Magenta Chromogen reagent (C30020-G###ML) with equal drops or parts (e.g. 200µl) of the Magenta Diluent reagent (C30021-G###ML) into a capped tube. Mix well by inverting WS several times prior to use.
3. For all assay applications, users should determine the appropriate incubation time for the Magenta WS prior to testing. An appropriate time to begin studies is 5 minutes for frozen tissues and 5-10 minutes for FFPE tissues. Depending upon preferences, users may elect to adjust incubation times by a minute to obtain lighter or darker stain results.
4. During test procedures, users should thoroughly wash off free HRP-antibody conjugates from tissues following both primary and/or secondary antibody incubation steps using a prepared 1X concentration of Novodiox ihc Wash Buffer (K52001-800) or similar PBS wash buffer. For best results, actively wash by shaking or moving slides up and down for 30-40 seconds.
5. When using the Novodiox pHRP antibodies, thoroughly wash following antibody incubation. Pipet sufficient Magenta WS to cover tissues, est. 100µl.

6. Following Magenta WS incubation step actively wash with either 1X concentration of ihc Wash Buffer, PBS or water.
7. As with any immunoassay, positive and negative controls should be run simultaneously with patient specimens.

User Recommendations:

1. Because of the reddish color, the ihc Magenta 1:1 dye can appear to be more sensitive than other chromogens like DAB. Extra washing following the antibody and chromogen steps can help to reduce the appearance of non-specific staining. For best results, actively wash by shaking or moving slides up and down for 30-40 seconds. Using multiple staining dishes/diptanks for wash buffers may also further help reduce background staining.
2. The chromogen will have a reddish color prior to mixing. As the WS is applied, users should see the reddish color on the tissue. This is normal and expected.
3. Since the ihc Magenta 1:1 dye yields a reddish-pink color, users may find that counterstains with a blue (Mayer's hematoxylin) or green (methyl green) hue provide a more optimal level of contrast.

Warnings and Precautions:

1. Read and understand all antibody test instructions prior to using this product in conjunction with an antibody reagent. It is helpful to test this product prior to reporting any results as the ihc Magenta 1:1 product may function better with certain cell types, e.g. cytoplasmic over others.
2. Take reasonable precautions when handling reagents. Use protective equipment such as disposable gloves and lab coats when handling suspected carcinogens or toxic materials. Read Safety Data Sheets (SDS) prior to use.
3. The kit should be stored at 2-8°C. Once mixed, the reagents are stable for 7 days when stored with a closed lid at 2-8°C.
4. Use caution and shorten incubation times when utilizing intense hematoxylin counterstains such as Gills as these stains may tend to mask antibody staining.
5. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
6. Patient specimens and all materials that come into contact with patient specimens should be handled as bio-hazardous materials and disposed of appropriately.
7. Consult local or state authorities with regard to recommended method of disposal of bio-hazardous and hazardous chemical waste materials.
8. Prepare and dispense mixed WS ihc Magenta 1:1 reagent. Do not attempt to add Magenta Chromogen reagent and Magenta Diluent separately.
9. Use lab grade quality chemicals such as acetone and water when preparing reagents.
10. Avoid microbial contamination of reagents.
11. Solutions containing sodium azide or other inhibitors of peroxidase activity should not be used in diluting the peroxidase substrate.

Unexpected Results:

If an unexpected staining pattern is observed on control tissues or tissue specimens which cannot be explained by variations in laboratory procedures or a problem with the ihc Magenta 1:1 Kit is suspected, contact Novodiox Technical Support or your local distributor immediately. Within the US call 1 (888) 439-2716 ext. 2 or 1 (510) 342-3043 ext. 2.

For technical support or further information about Novodiox test kits and products please contact Novodiox Technical Support or your local distributor.

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