Warp Red™ Chromogen Kit

Chromogen Kit
901-WR806-081017

BIOCARE MEDICAL

Catalog Number: **WR 806 H, S, M**

Description: 25 ml, 100 ml, 500 ml

Intended Use:
Warp Red™ Chromogen Kit consists of two solutions for the staining of formalinfixed, paraffin-embedded (FFPE) tissues, as part of an immunohistochemistry (IHC) procedure using an alkaline phosphatase (AP) detection system. The clinical interpretation of any staining or its absence should be complemented by morphological studies and 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:
Fast Red is a widely used chromogen for immunohistochemical staining. Warp Red is an advanced formulation of Fast Red that accelerates and intensifies staining. When in the presence of alkaline phosphatase (AP) enzyme, Warp Red produces a bright fuchsin-red precipitate that is insoluble in organic solvents and can be coverslipped with a permanent mounting media. Typically, Warp Red achieves staining intensity in 5-7 minutes that is equivalent to the staining previously observed with Vulcan Fast Red in 15-20 minutes. If users desire even more intense staining, Warp Red can be extended to 10 minutes. Warp Red is compatible with most alkaline phosphatase detection systems, including, MACH 2, MACH 3, MACH 4, 4plus, and PromARK. Warp Red may also be used in multiplex detection systems, such as MACH 2 Double Stain, for Multiplex IHC applications.

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues).

Supplied As:

25 ml size
1. Warp Red™ Chromogen (WR806CHE) 0.7 ml
2. Warp Red™ Buffer (WR806BFH) 25 ml

100 ml size
1. Warp Red™ Chromogen (WR806CHC) 2 x 1 ml
2. Warp Red™ Buffer (WR806BFL) 100 ml

500 ml size
1. Warp Red™ Chromogen (WR806CHC) 10 x 1 ml
2. Warp Red™ Buffer (WR806BFL) 5 x 100 ml

Materials and Reagents Needed But Not Provided:
Microscope slides, positively charged
Desert Chamber* (Drying oven)
Positive and negative tissue controls
Xylene (Could be replaced with a Xylene substitute*)
Ethanol or reagent alcohol
Decloaking Chamber* (Pressure cooker)
Deionized or distilled water
Wash buffer*(TBS)
Pretreatment reagents*
Enzyme digestion*
Avidin-Biotin Blocking Kit*(Labeled Streptavidin Kits Only)
Peroxidase block*
Protein block*
Primary antibody*
Negative control reagents*
Detection kits*
Hematoxylin*
Bluing reagent*
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.

Storage and Stability:
Store at 2°C to 8°C. Do not use after expiration date printed on vial. If reagents a restored 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 2°C to 8°C.

Protocol Recommendations:
Warp Red mixture should be prepared just prior to use. Apply the solution within 20 minutes of preparation.
1. Add 1 drop of Warp Red Chromogen to 2.5ml of Warp Red Buffer. Mix well.
2. Rinse tissue with TBS Wash Buffer.
3. Apply the Warp Red mixture to the tissue section. Incubate 5 to 7 minutes. 4. Rinse tissue with deionized water (D.I.).
5. Counterstain in hematoxylin. Rinse in D.I. water
6. Blue nuclei in TBS Wash Buffer for 1 minute. Wash in tap water.
7. Dehydrate rapidly in 3 changes of 100% alcohol and clear in 2 changes of xylene.
8. Mount and coverslip with permanent mounting medium.

Technical Note:
1. Warp Red Buffer may exhibit slight cloudiness; mix thoroughly before use.
2. Warp Red is highly fluorescent and will not fade. Use with a Texas Red filter.
3. Warp Red can be used with DAB for double stain procedures.
4. For increased staining intensity, the Warp Red application can be extended to 10 minutes.
5. Hydrogen peroxide block does not inhibit Warp Red staining and improves staining contrast.
6. Acetone can be used to reduce Warp Red overstaining.
7. Prolonged use of absolute alcohol or xylenes after Warp Red staining may cause fading.
8. If fading is observed, Biocare recommends to air dry slides after hematoxylin andbluing. Use Biocare's Desert Chamber drying oven at 60°C for 15-30 minutes. After drying, place slides in analytical grade xylene and coverslip.
9. When using an alkaline phosphatase system, Tris buffer (pH 7.6) should be used as a rinsing buffer. PBS should never be used! Phosphates act as a competitive inhibitor to alkaline phosphatase enzymes. For optimum performance, use Immunocare TBS Wash Buffer with AP activator or TBS Wash Buffer 20X.
10. To clean the mixing vial and/or dropper bottle, rinse with 70% alcohol and then wash in several changes of deionized water.

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. Warp Red Chromogen is mildly corrosive and may cause skin or eye irritation. Avoid contact with skin and eyes. If contact occurs, flush affected area with copious amounts of water. Seek medical attention if necessary.
2. Warp Red Buffer 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 or chromogen 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. Proclin™ is a trademark of Rohm and Haas Company, or of its subsidiaries or affiliates. Wear disposable gloves when handling reagents.
3. 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

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Precautions Cont'd:
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. (1)
4. Microbial contamination of reagents may result in an increase in nonspecific staining.
5. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
6. The SDS is available upon request and is located at http://biocare.net/.

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.

References:
1. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory workers from occupationally Acquired Infections; Approved guideline-Third Edition CLSI document M29-A3 Wayne, PA 2005.

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