

## Vulcan Fast Red Chromogen Kit 2

### Chromogen Kit

Control Number: 901-FR805-080515

ISO  
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**Catalog Number:** FR805 H, S, M, 5L

**Description:** 25 ml, 100 ml, 500 ml, 5L

#### Intended Use:

For In Vitro Diagnostic Use

Vulcan Fast Red Chromogen Kit 2 consists of two solutions for the staining of formalin-fixed, 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. When in the presence of alkaline phosphatase (AP) enzyme, Vulcan Fast Red produces a bright fuchsin-red precipitate that is insoluble in organic solvents--and can be coverslipped with a permanent mounting media. This product is available in a stable two-component system, consisting of Vulcan Fast Red Chromogen and Vulcan Buffer.

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.

#### Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

#### Supplied As:

##### 25 ml size

1. Vulcan Fast Red Chromogen (FR805CHE) 0.7 ml
2. Vulcan Fast Red Buffer (FR805BFH) 25 ml

##### 100 ml size

1. Vulcan Fast Red Chromogen (FR805CHC) 2 x 1 ml
2. Vulcan Fast Red Buffer (FR805BFL) 100 ml

##### 500 ml size

1. Vulcan Fast Red Chromogen (FR805CH) 5 x 1.6 ml
2. Vulcan Fast Red Buffer (FR805BFL) 5x100 ml

##### 5 L size

1. Vulcan Fast Red Chromogen (FR805CH-80) 80ml
2. Vulcan Fast Red Buffer (FR805BFO) 2x2.5L

#### 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 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 2°C to 8°C.

#### Protocol Recommendations:

Vulcan Fast Red mixture is stable for 20-30 minutes only. Solution should be made just prior to use.

1. Mix Vulcan Fast Red Chromogen gently by inverting several times.
2. Add 1 drop of Vulcan Fast Red Chromogen to 2.5ml of Vulcan Fast Red Buffer. Mix well.  
Note: To clean the mixing vial and/or dropper bottle, rinse with 70% alcohol and then wash in several changes of deionized water.
3. Rinse tissue with TBS Wash Buffer.
4. Apply the Vulcan Fast Red mixture to the tissue section. Incubate 10 to 15 minutes.
5. Rinse tissue with deionized (DI) water.
6. Counterstain in hematoxylin. Rinse in DI water.
7. Blue nuclei in TBS Wash Buffer for 1 minute. Wash in tap water.
8. Dehydrate rapidly in 3 changes of 100% alcohol and clear in 2 changes of xylene.
9. Mount and coverslip with permanent mounting medium.

#### Protocol Notes:

1. Vulcan Red is highly fluorescent and will not fade. Use with a Texas Red filter.
2. Vulcan Fast Red can be used with DAB for double stain procedures.
3. For increased sensitivity, a double application of Vulcan Fast Red (2 x 10 minutes) can be performed.
4. Hydrogen peroxide block does not inhibit Fast Red staining and improves staining contrast.
5. Acetone can be used to reduce Vulcan Fast Red overstaining.
6. Prolonged use of absolute alcohol after fast red staining may cause fading.
7. Vulcan Fast Red has been reported to fade in recycled alcohols, Fisher Histological Grade and Richard Allen alcohols.
8. Biocare recommends American Master Tech (Lodi California) Reagent Grade Alcohol (methanol free).
9. Biocare recommends to air dry slides after hematoxylin and bluing. Use Biocare's Desert Chamber drying oven at 60°C for 15-30 minutes.
10. After drying, place slides in analytical grade xylene and coverslip. If fading occurs use Biocare's EcoMount Mounting Media for coverslipping.
11. TBS wash buffers must be used for alkaline phosphatase procedures. PBS will dramatically reduce staining!
12. Coverslip in Biocare's EcoMount.

#### 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 intended for *in vitro* diagnostic (IVD) use.
2. Fast 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.
3. Fast 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.
4. 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. (1)

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**Precautions Cont'd:**

5. Microbial contamination of reagents may result in an increase in nonspecific staining.
6. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
7. 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-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.