



## Vulcan Fast Red Chromogen Kit 2

### Chromogen Kit

Control Number: 902-BRR805A-012611

ISO  
9001:2000  
CERTIFIED

**Catalog Number:** BRR 805A H,S,M,5L

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

**Intended Use:**

For Research Use Only. Not for use in diagnostic procedures

**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 (BRR805BE) 0.7 ml
2. Vulcan Fast Red Buffer (BRR805CH) 25 ml

**100 ml size**

1. Vulcan Fast Red Chromogen (BRR805BC) 2 x 1 ml
2. Vulcan Fast Red Buffer (BRR805CL) 100 ml

**500 ml size**

1. Vulcan Fast Red Chromogen (BRR805B) 5 x 1.6 ml
2. Vulcan Fast Red Buffer (BRR805CL) 5x100 ml

**5 L size**

1. Vulcan Fast Red Chromogen (BRR805B-80) 80ml
2. Vulcan Fast Red Buffer (BRR805CO) 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/PBS)
- 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 water (D.I.).
6. Counterstain in hematoxylin. Rinse in D.I. 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 degrees 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.

**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 BIOCARE 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.

**Quality Control:**

Refer NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information on Tissue Controls.

**Precautions:**

This product 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. Wear disposable gloves when handling reagents.

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. Microbial contamination of reagents may



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### Precautions Cont'd

result in an increase in nonspecific staining. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change. The MSDS is available upon request.

Consult OSHA, federal, state or local regulations for disposal of any toxic substances. Proclin™ is a trademark of Rohm and Haas Company, or of its subsidiaries or affiliates.

### 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.

### Limitations & Warranty:

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

