

Fluorescence Enhancement Probe (Rabbit)

Signal Amplification for Rabbit Primary Antibodies

Detection Component

Control Number: 901-FP003-082914

ISO
9001&13485
CERTIFIED

Catalog Number: FP003 G, H

Description: 6.0, 25 ml

Intended Use:

For In Vitro Diagnostic Use

Summary & Explanation:

BIOCARE's RABBIT fluorescent enhancement probe reacts specifically with Rabbit IgG (H +L) chains. This probe has been developed to significantly enhance the fluorescence staining intensity by increasing the sensitivity of detection when using rabbit monoclonal or polyclonal antibodies on paraffin sections, cryostat sections and cell culture preparations. Nuclear or cell membrane antigens that are weakly stained by indirect fluorescence methods will benefit from the increased signal provided by BIOCARE's fluorescent enhancement probe.

Known Applications:

Immunofluorescence on formalin-fixed paraffin-embedded tissue sections, cryostat sections and cell culture preparations.

Supplied As:

Yellow buffer with protein carrier and preservative.

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*

Mounting media*

* 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.

Species Reactivity:

Rabbit (H + L) chains

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:

Pretreatment Protocol:

Consider retrieving FFPE brain tissues in Reveal or DIVA at 80°C or 95°C for 45-60 minutes. Alternatively, steam tissue sections for 45-60 minutes. Allow solution to cool for 10-20 minutes then wash in distilled water. Fixed cryostat sections or cells cultured on slides may require a surfactant permeabilization step prior to antibody incubation.

Primary Antibody:

Titrate antibody on appropriate control tissue and incubate 60 minutes to 2 hours at room temperature, or overnight at 2-8°C.

Fluorescence Enhancement Probe:

Incubate for 15-30 minutes at RT.

Fluorescent Conjugate:

Incubate for 15-30 minutes at RT with DyLight™ goat anti-mouse 488 or 549 conjugate. Dilute fluor in BIOCARE's Fluorescence Antibody Diluent at the recommended dilution.

Nuclear Counterstain (optional):

DAPI or Hoechst dye for 3-5 minutes

Mountant:

Mount sections with appropriate anti-fade reagent. Consider using Biocare's Fluoro Care Anti-Fade Mountant*

Technical Notes:

Use PBS or TBS buffer to wash between steps.

Protocol Notes:

Protein blocking in human tissues can be performed with Biocare's Background Sniper for 10-15 minutes prior to antibody incubation. Consider using Biocare's Rodent Block M (RBM961) for mouse tissues or Rodent Block R (RBR962) for rat tissues if endogenous cross-reactivity is identified. Additional blocking with XR Factor (XRF964) in the working fluorescent conjugate has been shown to be effective at reducing cross-reactivity.

For technical assistance, please see troubleshooting.

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.

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Precautions:

Reagents contain less than 0.1% Sodium azide. Concentration less than 0.1% is not reportable hazardous material according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC.

Sodium azide (NaN₃) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center of Disease Control, 1976, National Institute of Occupational Safety and health, 1976).

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.

Minimize microbial contamination of reagents or increase in nonspecific staining may occur. 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.

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.

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