ERG + C4d

Prediluted Multiplex Antibody Reagent

Control Number: 901-3176DS-090817

Catalog Number: **API 3176DS AA**
Description: 6.0 ml, prediluted
Dilution: Ready-to-use
Diluent: N/A

Intended Use:
For In Vitro Diagnostic Use
ERG + C4d is a cocktail of mouse monoclonal and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of ERG and C4d proteins by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:

Tissue biopsy of transplanted organs remains the gold standard for evaluation of rejection or other pathologic causes of allograft dysfunction. In recent years, organ transplant pathology has evolved with recognition of antibody-mediated rejection (ABMR) in kidney and almost all transplanted solid organ systems. Immunohistochemical (IHC) analysis of C4d has a major role in diagnosis of ABMR. In ABMR, antibody binding to endothelial surfaces often activates the classic complement pathway via C1q. C1q activates C4, which is, in turn cleaved and activates C3, C5 and the membrane attack complex (1-4). This process damages endothelium, causes capillaritis and results in the recruitment of inflammatory cells to sites of complement activation (1-4). Although the inciting antibodies and other complement split products are degraded or carried away in the serum, the C4d fragment remains covalently bound to surfaces (via a thioester bond). Recent studies have focused on IHC scoring and interpretation of C4d staining to evaluate ABMR and correlating it with histologic findings, this often manifests as intracapillary inflammatory cells, termed capillaritis and immunostaining of C4d deposition in capillaries as a hallmark of antibody-mediated damage. However, activation of the complement via the mannose-binding lectin pathway also produces C4d; therefore, C4d staining is neither entirely specific nor perfectly sensitive (2-4). ERG, an ETS-family transcription factor is constitutively expressed in endothelial cells. It regulates endothelial cell differentiation, angiogenesis and expression of several endothelial-specific antigens and is also required for embryonic stem cells to differentiate into endothelial cells (5). Thus, the combination of C4d and ERG with a multiplexed membranous (C4d) and nuclear (ERG) staining pattern would facilitate the identification of the diseased endothelial cells and support a diagnosis of ABMR.

Principle of Procedure:

This product is a primary antibody cocktail of mouse and rabbit antibodies, which may be used in a Multiplex IHC staining procedure to produce a two-color stain. Following application of the primary antibody cocktail to the tissue sample, detection is performed by separate secondary antibodies specific for each species (i.e. mouse or rabbit) of the primary antibody cocktail, which are conjugated to horseradish peroxidase (HRP) or alkaline phosphatase (AP) enzymes. Visualization is accomplished by the application of chromogenic substrates (DAB and Warp Red), which are enzymatically activated (by HRP or AP, respectively) to produce a colored reaction product at the antigen site. The specimen may be counterstained and coverslipped. Results are interpreted using a light microscope.

Reagent Provided:
ERG + C4d is provided as a prediluted antibody cocktail of anti-ERG and anti-C4d antibodies in buffer with carrier protein and preservative.

Antibody	anti-ERG	anti-C4d
Clone	9FY	A24-T
Source	Mouse monoclonal	Rabbit monoclonal
Isotype	IgG1	IgG
Epitope/ Antigen	N-terminal ERG	Internal sequence of human C4
Cellular Localization	Nuclear	Basement membrane/ cytoplasm
Staining	Brown (DAB)	Red (Warp Red)

Storage and Stability:
Store at 2ºC to 8ºC. Do not use reagent after the expiration date printed on the vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user.

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

Species Reactivity: Human; others not tested

Positive Tissue Control: Renal allograft tissue

Protocol Recommendations:

Peroxide Block: Block for 5 minutes with Biocare's Peroxidazed 1.

Pretreatment: Perform heat retrieval using Biocare's Diva Decloaker. Refer to the Diva Decloaker product data sheet for specific instructions.

Protein Block: Incubate for 10 minutes at RT with Biocare's Background Punisher.

Primary Antibody: Incubate for 30 minutes at RT.

Double Stain Detection: Incubate for 30 minutes at RT using Biocare's MACH 2 Double Stain 2.

Chromogen (1): Incubate for 5-7 minutes at RT with Biocare's Warp Red. Rinse in deionized water.

Chromogen (2): Incubate for 5 minutes at RT with Biocare's DAB.

Counterstain: Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Technical Notes:

This antibody has been standardized with Biocare's MACH 2 Double Stain 2. Use TBS buffer for washing steps.

Limitations:

The optimum antibody dilution and 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 antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN_3) 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 for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (6)
2. 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.

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ISO 9001&13485 CERTIFIED

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Precautions Cont'd:

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. (7)

3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at http://biocare.net.

Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

References:

1. Feucht HE. Complement C4d in graft capillaries—the missing link in the recognition of humoral alloreactivity. Am J Transplant. 2003; 3:646–52.
2. Cohen D, *et al*. Pros and cons for C4d as a biomarker. Kidney Int. 2012; 81:628–39.
3. Nickeleit V, Mihatsch MJ. Kidney transplants, antibodies and rejection: is C4d a magic marker? Nephrol Dial Transplant. 2003; 18:2232–39.
4. Bohmig G, Regele H. Diagnosis and treatment of antibody-mediated kidney allograft rejection. Transpl Int. 2003; 16:773–87.
5. Miettinen M, *et al*. ERG expression in epithelioid sarcoma: a diagnostic pitfall. Am J Surg Pathol 2013; 37:1580–5.
6. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
7. 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.

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