

ERCC1

Concentrated Monoclonal Antibody
901-3147-090117

BIOCARE
M E D I C A L

Catalog Number: ACI 3147 A, B

Description: 0.1, 0.5 ml, concentrated

Dilution: 1:100

Diluent: Renoir Red

Intended Use:

For In Vitro Diagnostic Use

ERCC1 [4F9] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of ERCC1 protein 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:

The excision repair cross-complementation group 1 (ERCC1) gene encodes a protein required for nucleotide excision repair and inter-strand crosslink repair of DNA (1). While DNA-damaging chemotherapeutic drugs like cisplatin are commonly used for non-small cell lung carcinoma and other late-stage tumors, these drugs have a high degree of resistance and severe side effects (1,3). Platinum chemotherapy drug resistance has been linked to elevated levels of ERCC1-XPF nuclease, therefore making ERCC1 a potential predictive diagnostic biomarker (2,3). ERCC1 expression may have prognostic value in lung, colorectal, head and neck, bladder, breast and cervical cancers (1-7).

Although clone 8F1 has traditionally been used in immunohistochemistry to detect ERCC1 expression, 8F1 has been found to cross-react with PCYT1A, an unrelated nuclear membrane protein. Clone 4F9 does not show this cross-reaction, providing superior specificity for ERCC1 expression (2-4).

Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

Source: Mouse monoclonal

Species Reactivity: Human; others not tested

Clone: 4F9

Isotype: IgG1

Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration.

Epitope/Antigen: Protein expressed in 293T cell transfected with human ERCC1 expression vector

Cellular Localization: Nuclear, cytoplasmic

Positive Tissue Control: Prostate or prostate cancer

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative

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:

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

Protocol Recommendations Cont'd:

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

Protein Block (Optional): Incubate for 5-10 minutes at RT with Biocare's Background Punisher.

Primary Antibody: Incubate for 30 minutes at RT.

Probe: Incubate for 10 minutes at RT with a secondary probe.

Polymer: Incubate for 10-20 minutes at RT with a tertiary polymer.

Chromogen:

Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Biocare's Warp Red.

Counterstain:

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

Technical Note:

This antibody has been standardized with Biocare's MACH 4 detection system. 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 (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 for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (7)
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. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (8)
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.

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Precautions Cont'd:

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. Bhagwat NR, *et al.* Immunodetection of DNA repair endonuclease ERCC1-XPF in human tissue. *Cancer Res.* 2009 Sep 1;69(17):6831-8.
2. Ma D, *et al.* Using protein microarray technology to screen anti-ERCC1 monoclonal antibodies for specificity and applications in pathology. *BMC Biotechnol.* 2012 Nov 21;12:88.
3. Smith DH, *et al.* Measuring ERCC1 protein expression in cancer specimens: validation of a novel antibody. *Sci Rep.* 2014 Mar 7;4:4313.
4. Bauman JE, *et al.* ERCC1 is a prognostic biomarker in locally advanced head and neck cancer: results from a randomised, phase II trial. *Br J Cancer.* 2013 Oct 15;109 (8):2096-105.
5. Ozcan MF, *et al.* Low ERCC1 expression is associated with prolonged survival in patients with bladder cancer receiving platinum-based neoadjuvant chemotherapy. *Urol Oncol.* 2013 Nov;31(8):1709-15.
6. Palomba G, *et al.* ERCC1 polymorphisms as prognostic markers in T4 breast cancer patients treated with platinum-based chemotherapy. *J Transl Med.* 2014 Sep 25;12:272.
7. 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."
8. 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.