

PHOSPHO-EGFR

Prediluted Rabbit Monoclonal Antibody
Control Number: 901-300-093014

ISO
9001&13485
CERTIFIED

Catalog Number: API 300 AA
Description: 6.0 ml, prediluted
Dilution: Ready-to-use
Diluent: N/A

Intended Use:

For In Vitro Diagnostic Use
PHOSPHO-EGFR [EP774Y] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of epidermal growth factor receptor protein phosphorylated at Tyr1068 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:

Epidermal Growth Factor Receptor (EGFR) is a 170kDa transmembrane glycoprotein receptor tyrosine kinase and is activated by EGF. This activation affects cell growth and differentiation. The carboxy terminal tyrosine residues on EGFR, Tyr1068, Tyr1148 and Tyr1173, are major sites of autophosphorylation, which occurs as the result of EGF binding. Once activated, phosphotyrosines mediate the binding of growth factor receptor-binding protein-2 (Grb2) to the EGFR. This antibody only detects EGFR phosphorylated on Tyrosine 1068 of the mature human isoform that corresponds to Y1092 from the precursor form P00533-1(p170). Over-expression of EGFR has been reported in tumors of breast, lung, colon, cervix, ovary, esophagus and endometrium.

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, an enzyme labeled polymer is added to bind to the primary antibody. The detection of the bound antibody is evidenced by a colorimetric reaction.

Source: Rabbit monoclonal

Species Reactivity: Human, mouse

Clone: EP774Y

Isotype: IgG

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

Epitope/Antigen: Phospho-EGFR (Tyr1068)

Cellular Localization: Membrane and / or cytoplasmic

Positive Control: Squamous cell carcinoma or colon cancer

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative
Van Gogh Yellow (PD902)

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.

Protocol Recommendations:

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

Pretreatment Solution (recommended): Diva

Pretreatment Protocol:

Heat Retrieval Method:

Retrieve sections under pressure using Biocare's Decloaking Chamber, followed by a wash in distilled water; alternatively, steam tissue sections for 45-60 minutes. Allow solution to cool for 10 minutes then wash in distilled water.

Protocol Recommendations Cont'd:

Protein Block (Optional): Incubate for 10-15 minutes at RT with Biocare's Background Eraser*.

Primary Antibody: Incubate for 30 minutes at RT.

Probe: N/A

Polymer: Incubate for 30 minutes at RT with a secondary-conjugated 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 2 detection system. It can also be used on an automated staining system and with other Biocare polymer detection kits. Use TBS buffer for washing steps.

*Do not use Sniper based diluents or blockers, as staining will be reduced by 50%.

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 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 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. 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 expiration date printed on the vial.
6. The SDS is available upon request and is located at <http://biocare.net/>.

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References:

1. Cornianu M, Tudose N. Immunohistochemical markers in the morphological diagnosis of lung carcinoma. Rom J Morphol Embryol. 1997 Jul-Dec;43(3-4):181-91.
2. Bue P, *et al.* Expression of epidermal growth factor receptor in urinary bladder cancer metastases. Int J Cancer. 1998 Apr 13;76(2):189-93.
3. Mansour OA, *et al.* Epidermal growth factor receptors: status and effect on breast cancer patients. Anticancer Res. 1997 Jul-Aug;17(4B):3107-10.
4. Mansour OA, *et al.* Tissue and serum c-erbB-2 and tissue EGFR in breast carcinoma: three years follow-up. Anticancer Res. 1997 Jul-Aug;17(4B):3101-6.
5. Willsher PC, *et al.* Male breast cancer: pathological and immunohistochemical features. Anticancer Res. 1997 May-Jun;17(3C):2335-8.
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.

Manufactured incorporating RabMAb® technology under Epitomics US Patents, No 5,675,063 and 7,429,487, owned by Abcam.

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.

