



PHOSPHO-EGFR

Concentrated and Prediluted Monoclonal Antibody

Control Number: 902-300-032708

Catalog Number:	CME 300 AK,BK	PME 300 AA
Description:	0.1, 0.5ml, concentrated	6.0 ml, prediluted
Dilution:	1:100-1:200	Ready-to-use
Diluent:	Van Gogh Yellow	N/A

Intended Use:
For Research Use Only

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, a universal, affinity-purified, 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: Rabbit Monoclonal

Species Reactivity: Human and Mouse

Clone: EP774Y

Isotype: Rabbit IgG

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

Epitope/Antigen: EGFR

Cellular Localization: Membrane and/or cytoplasmic

Positive Control: Squamous cell carcinoma or colon cancer

Normal Tissue: Skin

Abnormal Tissue: Colon, squamous cell and basal cell carcinomas

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. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations

Peroxide Block:

If using an HRP system, block for 5 minutes with BIOCARE's PEROXIDAZED 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 20 minutes then wash in distilled water.

Protein Block:

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 Polymer.

Chromogen:

Incubate for 5 minutes at RT when using BIOCARE's DAB. - OR - Incubate for 10 minutes at RT when using BIOCARE's Vulcan Fast Red.

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%.

Performance Characteristics:

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

Precautions:

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)

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 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.

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.

Limitations and 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.

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.





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References cont'd:

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. National Committee for Clinical Laboratory Standards(NCCLS). Protection of laboratory workers from infectious diseases transmitted by blood and tissue; proposed guideline. Villanova, PA 1991;7(9). Order code M29-P.

This antibody was produced using EPITOMICS proprietary rabbit monoclonal antibody technology (U.S. Patent No. 5,675,063).

