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Product datasheet for AM00031PU-N

EGFR pTyr869 (incl. pos. control) Mouse Monoclonal Antibody [Clone ID: 12A3]

Product data:

Product Type:	Primary Antibodies
Clone Name:	12A3
Applications:	ELISA, IF, IHC, IP, WB
Recommended Dilution:	ELISA: Use at 0.1 µg/ml (direct ELISA). Immunocytochemistry: Use at 1-10 µg/ml. Immunohistochemistry on Frozen and Paraffin Embedded Material. Immunoprecipitation: Use at 1-10 µg per 10e6 vanadate-treated A431 cells. Western Blot: 1 µg/ml for HRPO/ECL detection. Recommended buffer: Casein/Tween 20 based blocking and blot incubation buffer (cat# AS00002BU-N). *Included Positive Control:* Cell lysate delivered with this product (See Protocol below!).
Reactivity:	Human, Mouse
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Phosphopeptide conjugated to KLH Epitope: Phosphotyrosine 869
Specificity:	Clone 12A3 specifically recognizes EGFR phosphorylated at Tyrosine 869 and detects EGFR activation after interaction with src kinases. Antibody AM00031PU-N does not crossreact with the highly homologues pTyr 877 of activated erbB2.
Formulation:	PBS containing 0.09% Sodium Azide / PEG and Sucrose. State: Purified State: Lyophilized purified Ig fraction.
Reconstitution Method:	Restore in 1 ml distilled water for 15 min at RT.
Purification:	Subsequent Thiophilic Adsorption and Size Exclusion Chromatography.
Conjugation:	Unconjugated

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Storage: For long-term storage, freeze lyophilizate upon arrival (-20°C). Upon reconstitution, aliquote and freeze in liquid nitrogen; reconstituted antibody can be stored frozen at -80°C up to 1 year. Thaw aliquots at 37°C. Thawed aliquots may be stored at 2-8°C up to 3 months.
Avoid repeated freeze / thaw cycles!

Database Link: Entrez Gene 1956 Human
P00533

Background: EGFR/erbB receptors are activated upon binding of EGF and EGF-related growth factors such as TGF alpha, beta-cellulin, Hb-EGF, HRG, or NRG. Binding of these ligands leads to receptor homo- and heterodimerization followed by autophosphorylation and activation of downstream signal transduction pathways (MAPK, PI3K/PKB, and STAT). In addition, EGFR becomes fully activated after phosphorylation of Y869 by src family kinases. Phosphorylation of Y1069 leads to association with cbl and subsequent receptor degradation. Phosphorylation of S1071 by CamKinase II leads to attenuation of kinase activity; phosphorylation of T678 (by PKC) and T693 (by MAPK, p38) interferes with receptor endocytosis/recycling.

Synonyms: Epidermal growth factor receptor, EGF Receptor, erbB-1, c-ErbB-1

Note: **Mol. weight of antigen:** 180 kD.
A positive Control is provided (for details see protocol below).

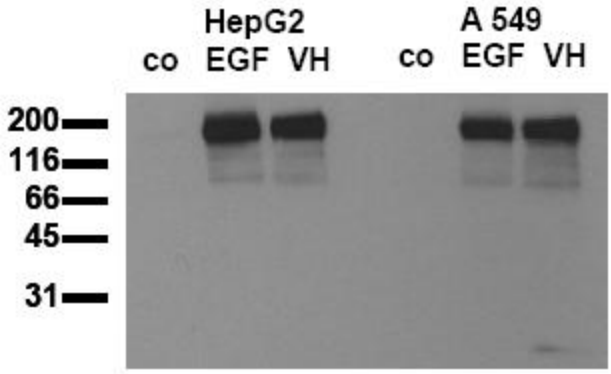
Protocol: **Positive control cell lysate provided:**
HepG2 EGF Treated - recommended for immunoblotting

Formulation:
Lyophilized cell lysate from HepG2 cells. Serum starved cells were treated for 15 min. with EGF.
Restore by addition of 200 µl H2O. After complete solubilization add 200 µl 2x SDS-PAGE sample buffer, mix and incubate at 90°C for 5 min. Store in aliquots at -20°C. Avoid repeated freezing and thawing.

Applications:
20 µl of positive cell lysate correspond to ca. 80.000 cells. Use 20 µl / lane (mini gel) for HRPO/ECL detection of the target proteins.

Please note:
The lyophilized cell lysate contains SDS and is not recommended for applications with native proteins such as immunoprecipitation.

Product images:



Phosphospecificity: Whole cell extracts of control (co), EGF stimulated (EGF) or pervanadate treated (VH) HepG2 and A549 tumor cells were applied to SDS-PAGE (ca 20.000 cells per lane) and transferred to a PVDF membrane. The immunoblot was probed with mab EGFR-12A3 (0.5 ug/ ml) for 1h at RT and developed by ECL (exp. time: 30 sec).