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Datasheet for 100-401-149

EGFR Antibody

Overview

Description:	Anti-EGFR (RABBIT) Antibody - 100-401-149
Item No.:	100-401-149
Size:	250 µL
Applications:	IHC, IP, WB
Reactivity:	Human, Rat
Host Species:	Rabbit

Product Details

Background:	EGFR is a transmembrane glycoprotein that is a member of a family of protein tyrosine kinases crucial to maintaining a normal balance in cell growth and development. Growth factor receptors are involved not only in promoting the proliferation of normal cells but also in the aberrant growth of many types of human tumors. For example, the epidermal growth factor receptor (EGFR) is mutated and/or over-expressed in many common solid human squamous cell carcinomas including breast, brain, bladder, lung, gastric, head & neck, esophagus, cervix, vulva, ovary, and endometrium. Over-expression of the EGFR gene occurs in carcinomas with and without gene amplification. EGFR and ErbB-2 are particularly important in breast cancer because increased production or activation has been associated with poor prognosis. EGFR belongs to a family of growth factor receptors, which also includes ErbB-2/HER-2/neu, ErbB-3/HER-3/neu and ErbB-4/HER-4/neu. EGFR can heterodimerize with each of the members of this family.
Synonyms:	rabbit anti-EGFR Antibody, rabbit anti-epidermal growth factor receptor antibody, Receptor tyrosine-protein kinase erbB-1 antibody, c-erbB-1 antibody
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	Antiserum

Target Details

Gene Name:	EGFR
Reactivity:	Human, Rat

Immunogen Type:	Conjugated Peptide
Immunogen:	This whole rabbit serum was prepared by repeated immunizations with a peptide synthesized using conventional technology. The sequence of the epitope maps to a region near the carboxy terminus which is identical in human, mouse and rat EGFR.
Purity/Specificity:	This antiserum is directed against human epidermal growth factor receptor (EGFR) and is useful in determining its presence in western blotting and immunoprecipitation experiments. This antibody can detect EGFR from human, mouse and rat sources. Reactivity of this antibody with EGFR from other species is unknown. No reaction is observed against ErbB-2, ErbB-3 or ErbB-4.
Relevant Links:	• UniProtKB - P00533 • NCBI - 29725609 • GeneID - 1956

Application Details

Tested Applications:	IHC, IP, WB
Application Note:	Anti-EGFR antibody has been tested by and is specifically designed for ELISA, immunoblotting, immunoprecipitation, and immunohistochemistry. Reactivity in other assays is likely, but has not been determined. Recognition of EGFR is independent of the phosphorylation status at tyrosine 1173. A431 cells, keratinocytes in normal epidermis, or placenta are typically used as positive control sources. The antigen is typically localized in the cell membrane. For western blotting, good results are also achieved on PVDF membranes blocked with 5% lowfat milk diluted in TTBS for 1 hour at room temperature. Also, dilute the primary antibody and secondary in 5% lowfat milk in TTBS. Anti-EGFR can be diluted up to 1:10,000 for immunoblot depending on the cell line and the amount of EGFR in a particular lysate. For immunoprecipitation, use approximately 10 µl of the antibody. The immunoprecipitation mix should contain the antibody, 25 µl of Protein A-agarose beads and 1.0 ml of lysate (lysate contains approximately 1.0 mg of total protein). This mixture should be rotated overnight at 4°C and then washed 3 times with lysis buffer (used to prepare the lysate). The resulting bead complex is dissolved in 20-30 µl of 3X SDS-PAGE sample buffer and approximately 15 µl is loaded per lane on an 8% polyacrylamide gel.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 1:50,000
IHC:	2.5 µg/mL
IP:	10 µl
WB:	1:1,000 - 1:10,000

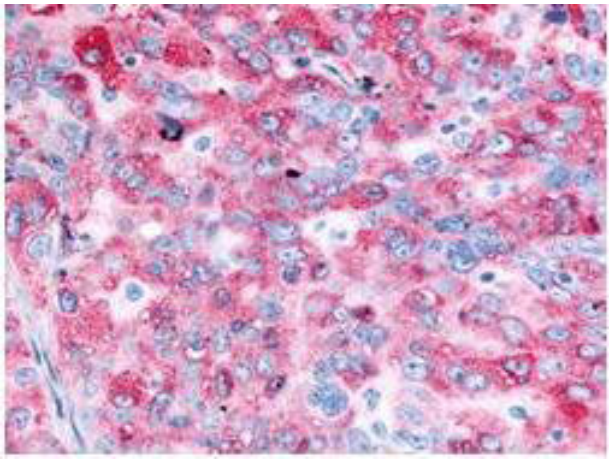
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	85 mg/mL by Refractometry
Buffer:	None
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

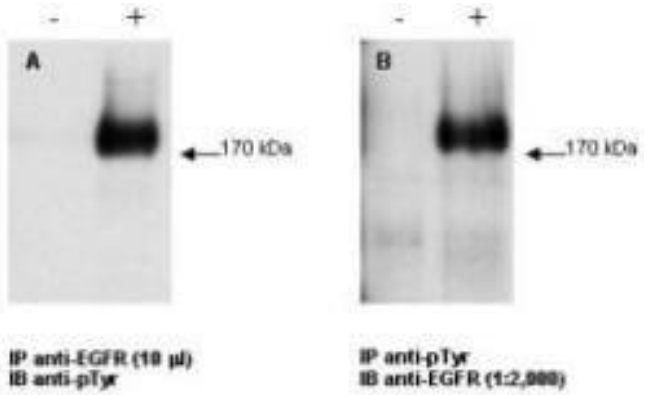
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



Immunohistochemistry
Immunohistochemistry of Anti-EGFR Antibody with positive staining.



Western Blot
Combined immunoprecipitation and western blot using anti-EGFR antibody. Lysates were prepared from GN4 rat liver epithelial cells both with (+) EGF treatment for 15' at 100 ng/ml and without (-) the addition of EGF. The combination of immunoprecipitation and western blotting was performed using the anti-EGFR antibody for immunoprecipitation (10 µL) followed by western blot detection using an anti-phosphotyrosine antibody (Panel A). This was repeated in reverse order using a 1:2000 dilution of anti-EGFR for western blot (Panel B). Visualization occurred using an ECL system. Film exposure was approximately 1'. Other detection systems will yield similar results.

Western Blot
Western blot using Rockland's anti-EGFR antibody. Lane 1: unstimulated A431 whole cell lysates (p/n W09-000-361). Lane 2: EGF stimulated A431 whole cell lysates (p/n W09-00-362). Shows detection of a band at ~170 kDa corresponding to human EGFR present in unstimulated and stimulated (50 ng/ml for 15 min) lysates (arrowhead). Loaded: 30µg lysate was resolved on a 4-20% Tris-Glycine gel by SDS-PAGE and transferred onto nitrocellulose. Primary Antibody: Anti-EGFR at 1:1,000 overnight at 4° C. Secondary Antibody: IRDye® 800 conjugated Gt-a-Rabbit IgG (H&L) MX10 (p/n 611 -132-122) at 1:10,000 dilution of for 45 min at room temperature (800 nm channel, green). Molecular weight estimation was made by comparison to prestained MW markers in lane M (700 nm channel, red). IRDye® 800 fluorescence image was captured using the Odyssey® Infrared Imaging System developed by LI-COR. IRDye is a trademark of LI-COR, Inc. Other detection systems will yield similar results.

References

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