Datasheet for ABIN98862

anti-EGFR antibody

1 Validation | 2 Images | 3 Publications

Go to Product page

Overview

Quantity:	250 µL
Target:	EGFR
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This EGFR antibody is un-conjugated
Application:	Western Blotting (WB), ELISA, Immunoprecipitation (IP)

Product Details

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.
Characteristics:	Concentration Definition: by Refractometry

Target Details

Target:	EGFR
Alternative Name:	EGFR (EGFR Products)
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

Target Details

	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: Receptor tyrosine-protein kinase erbB-1 antibody, c-erbB-1 antibody
Gene ID:	1956, 29725609
UniProt:	P00533
Pathways:	NF-kappaB Signaling, RTK Signaling, Fc-epsilon Receptor Signaling Pathway, EGFR Signaling Pathway, Neurotrophin Signaling Pathway, Stem Cell Maintenance, Hepatitis C, Positive Regulation of Response to DNA Damage Stimulus, Interaction of EGFR with phospholipase C-gamma, Thromboxane A2 Receptor Signaling, EGFR Downregulation, S100 Proteins

Application Details

Application Notes:	Anti-EGFR antibody is specifically designed for ELISA, immunoblotting and immunoprecipitation. Reactivity in other assays is likely, but has not been determined. Recognition of EGFR is independent of the phosphorylation status at tyrosine 1173. No reaction is observed against ErbB-2, ErbB-3 or ErbB-4. 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.
Restrictions:	For Research Use only

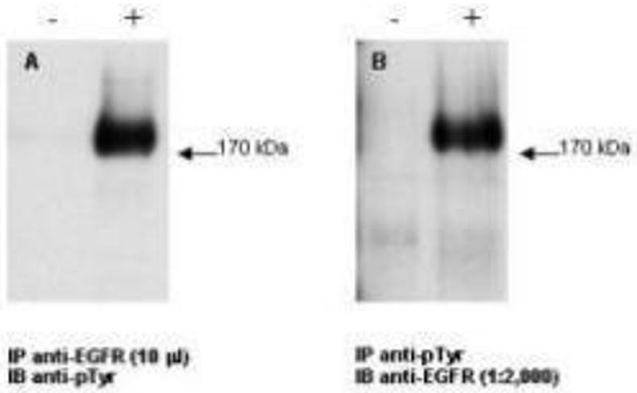
Handling

Format:	Liquid
Concentration:	85 mg/mL
Preservative:	Sodium azide
Precaution of Use:	This product contains sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	-20 °C

Publications

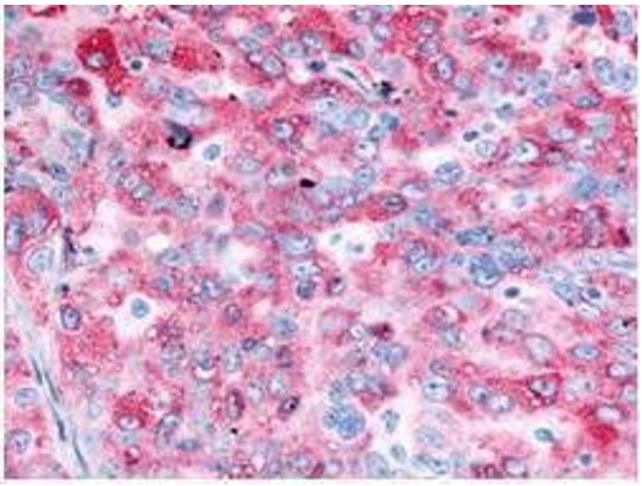
Product cited in:	Muñoz, Beltrán-Alzate, Duthie, Serrano-Coll, Cardona-Castro: "Comparison of Enzyme-Linked Immunosorbent Assay Using Either Natural Octyl Disaccharide-Leprosy IDRI Diagnostic or Phenolic Glycolipid-I Antigens for the Detection of Leprosy Patients in Colombia." in: **The American journal of tropical medicine and hygiene**, Vol. 98, Issue 1, pp. 274-277, (2018) (PubMed).

Validation report #101369 for Immunofluorescence (IF)



Western Blotting

Image 1. 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.



Immunohistochemistry

Image 2. 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.

Validation report #029817 for Western Blotting (WB)

Successfully validated (Western Blotting (WB))

by ADS Biosystems Inc

Report Number: 029817

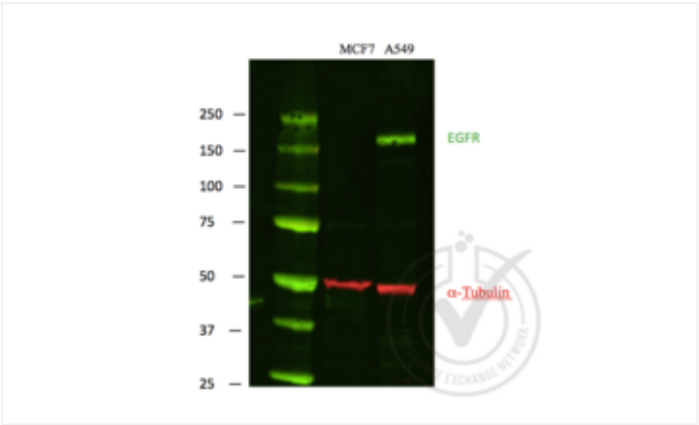
Date: Sep 18 2014

Lot Number:	19538
Method validated:	Western Blotting (WB)
Positive Control:	A549 cells
Negative Control:	MCF-7 cells
Notes:	A strong specific band was observed in the positive control at the expected size (~175 kDa) that is not observed in the negative control.
Primary Antibody:	- Antigen: Epidermal Growth Factor Receptor (EGFR) - Catalog number: ABIN98862 - Supplier: antibodies-online - Lot number: 19538 - Dilution: 1:1,000
Secondary Antibody:	- Antibody: IRDye 680LT Goat Anti-Rabbit - Catalogue number: 827-11081 - Supplier: LI-COR Biosciences - Lot number: C30725-01 - Dilution: 1:10,000
Controls:	- Lysates were prepared by ADS Biosystems following standard protocols and quality controlled for protein integrity on a regular basis
Protocol:	• Lysates were mixed with NuPAGE® LDS Sample Buffer (Life Technologies NP0007) and NuPAGE® Sample Reducing Agent (Life Technologies NP0004) and denatured for 5 minutes at 90°C. • 40 µg of each lysate was electrophoresed on a Bolt 4-12% Bis-Tris Gel (Life Technologies BG04120BOX) and run in Bolt MOPS SDS Running Buffer (Life Technologies B0001) at 160 volts for 1 hour. • Odyssey Western Protein Standard (LI-COR #928-40000) was run as a molecular weight standard. • PVDF membrane was activated with methanol. • Protein samples were transferred to activated PVDF membrane in a wet Bolt Transfer Apparatus (Life Technologies B1000) at room temperature for 1 hour at 20 volts (started at 230mA, ended at 110mA). • The membrane was blocked in x LI-COR Odyssey WB block solution for 1 hour at room temperature. • The membrane was incubated with the primary antibody diluted 1:1000 in x LI-COR Odyssey WB block solution incubated 2 hours at room temperature. • The membrane was washed 4 x 5 minutes in 1 x PBS-T (PBS solution with 0.1% Tween 20).

- The membrane was incubated with IRDye® 800CW Goat anti-Mouse Secondary Antibody (Red) and IRDye 680LT Goat Anti-Rabbit Secondary Antibody (Green) from LI-COR (#827-11081, Lot #C30725-01), both 1:10,000 dilutions. Incubation was performed at room temperature for 45 minutes.
- The membrane was washed 4 x 5 minutes in 1 x PBS-T (PBS solution with 0.1% Tween 20).
- Proteins were detected using Odyssey machine scanning with green channel for loading control and red channel for potential LPL band.

Experimental Notes:	- No experimental challenges noted.

Image for Validation report #029817



Validation image no. 1 for anti-Epidermal Growth Factor Receptor (EGFR) antibody (ABIN98862)

Figure 1: Scanned image of EGFR (Green) and loading control alpha-tubulin (Red) Western blot using LI-COR Odyssey Infrared Technology. First lane, protein molecular weight markers. Second lane, MCF-7 negative control lysate. Third lane, A549 positive control lysate.