

Product datasheet

Anti-ErbB 2 antibody [EPR19547-12] ab214275

Recombinant **RabMAb**

★★★★★ 2 Abreviews 11 Images

Overview

Product name	Anti-ErbB 2 antibody [EPR19547-12]
Description	Rabbit monoclonal [EPR19547-12] to ErbB 2
Host species	Rabbit
Specificity	Only reacts with mouse weakly in 4T1 cells
Tested applications	Suitable for: ICC/IF, IP, Flow Cyt, IHC-P, WB
Species reactivity	Reacts with: Rat, Human
Immunogen	Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) within Human ErbB 2 aa 1200 to the C-terminus (phospho Y1248). The exact sequence is proprietary. (ab214275 recognizes both phospho and non-phospho ErbB2). Database link: P04626
Positive control	WB: Human ErbB 2 fragment recombinant protein; human breast cancer lysate; HeLa, A431, SK-BR-3, C6 and PC-12 whole cell lysates. IHC-P: Human breast cancer tissue. ICC/IF: SK-BR-3 cells. Flow Cyt: SK-BR-3 cells. IP: HeLa whole cell lysate.
General notes	Our RabMAb [®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents . This product is a recombinant rabbit monoclonal antibody .

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR19547-12
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab214275** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★★★	1/250.
IP		1/30.
Flow Cyt		1/60. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★★★★☆	1/1000. Detects a band of approximately 180 kDa (predicted molecular weight: 137 kDa).

Target

Function

Protein tyrosine kinase that is part of several cell surface receptor complexes, but that apparently needs a coreceptor for ligand binding. Essential component of a neuregulin-receptor complex, although neuregulins do not interact with it alone. GP30 is a potential ligand for this receptor. Regulates outgrowth and stabilization of peripheral microtubules (MTs). Upon ERBB2 activation, the MEMO1-RHOA-DIAPH1 signaling pathway elicits the phosphorylation and thus the inhibition of GSK3B at cell membrane. This prevents the phosphorylation of APC and CLASP2, allowing its association with the cell membrane. In turn, membrane-bound APC allows the localization of MACF1 to the cell membrane, which is required for microtubule capture and stabilization. In the nucleus is involved in transcriptional regulation. Associates with the 5'-TCAAATTC-3' sequence in the PTGS2/COX-2 promoter and activates its transcription. Implicated in transcriptional activation of CDKN1A; the function involves STAT3 and SRC. Involved in the transcription of rRNA genes by RNA Pol I and enhances protein synthesis and cell growth.

Tissue specificity

Expressed in a variety of tumor tissues including primary breast tumors and tumors from small bowel, esophagus, kidney and mouth.

Involvement in disease

Hereditary diffuse gastric cancer
Glioma
Ovarian cancer
Lung cancer
Gastric cancer
Chromosomal aberrations involving ERBB2 may be a cause gastric cancer. Deletions within 17q12 region producing fusion transcripts with CDK12, leading to CDK12-ERBB2 fusion leading to truncated CDK12 protein not in-frame with ERBB2.

Sequence similarities

Belongs to the protein kinase superfamily. Tyr protein kinase family. EGF receptor subfamily. Contains 1 protein kinase domain.

Post-translational modifications

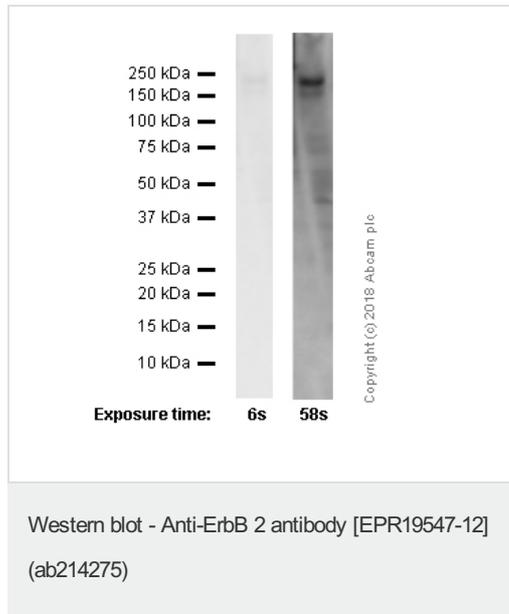
Autophosphorylated. Autophosphorylation occurs in trans, i.e. one subunit of the dimeric receptor phosphorylates tyrosine residues on the other subunit (Probable). Ligand-binding increases phosphorylation on tyrosine residues (PubMed:27134172). Signaling via SEMA4C promotes

phosphorylation at Tyr-1248 (PubMed:17554007). Dephosphorylated by PTPN12 (PubMed:27134172).

Cellular localization

Cytoplasm. Nucleus and Cell membrane. Cytoplasm, perinuclear region. Nucleus. Translocation to the nucleus requires endocytosis, probably endosomal sorting and is mediated by importin beta-1/KPNB1.

Images



All lanes : Anti-ErbB 2 antibody [EPR19547-12] (ab214275) at 1/1000 dilution

Lane 1 : 4T1 (Mouse mammary gland carcinoma epithelial cell) whole cell lysate

Lane 2 : 4T1 (Mouse mammary gland carcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

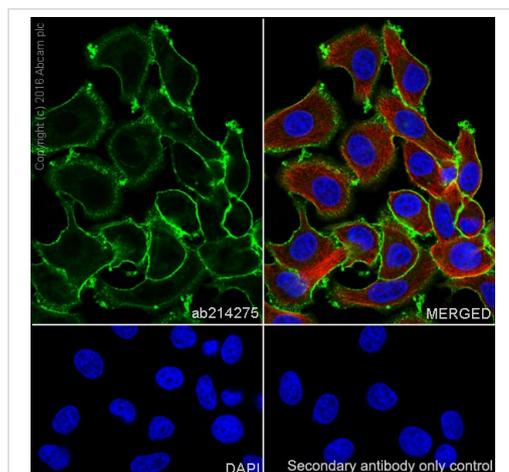
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 137 kDa

Observed band size: 180 kDa

[why is the actual band size different from the predicted?](#)

Blocking and diluting buffer: 5% NFDm/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-ErbB 2 antibody [EPR19547-12] (ab214275)

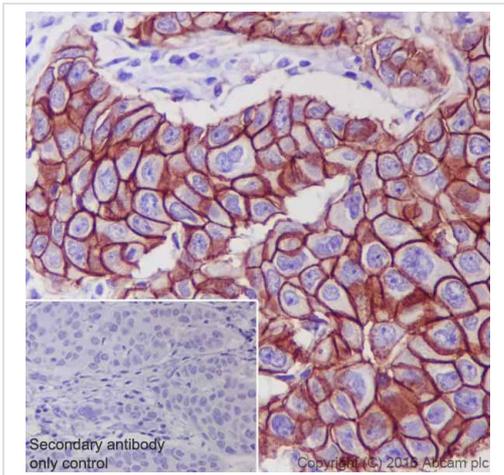
Immunofluorescent analysis of 100% methanol-fixed SK-BR-3 (Human mammary gland adenocarcinoma cell line) cells labeling ErbB 2 with ab214275 at 1/250 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Confocal image showing membrane staining on SK-BR-3 cells.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution.



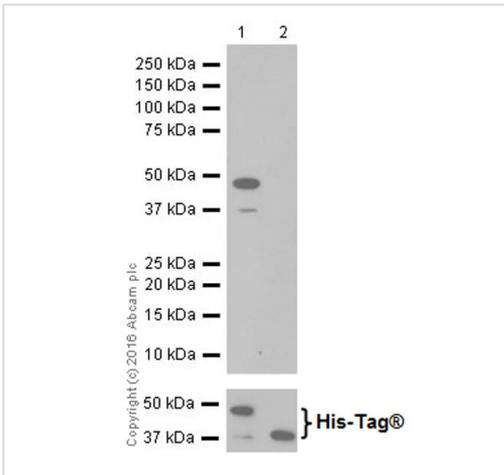
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ErbB 2 antibody [EPR19547-12] (ab214275)

Immunohistochemical analysis of paraffin-embedded Human breast cancer tissue labeling ErbB 2 with ab214275 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Membrane staining on Human breast cancer is observed [PMID: 18437174].

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab97051 at 1/500 dilution.



Western blot - Anti-ErbB 2 antibody [EPR19547-12] (ab214275)

All lanes : Anti-ErbB 2 antibody [EPR19547-12] (ab214275) at 1/1000 dilution

Lane 1 : Human ErbB 2 fragment recombinant protein

Lane 2 : Human ErbB 4 fragment recombinant protein

Lysates/proteins at 0.02 µg per lane.

Secondary

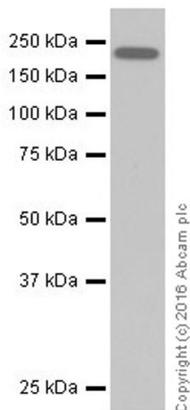
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 137 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

Human ErbB 2 fragment recombinant protein contains aa955-1255 with a His-Tag®; Human ErbB 4 fragment recombinant protein contains aa1000-1300 with a His-Tag®.



Western blot - Anti-ErbB 2 antibody [EPR19547-12] (ab214275)

Anti-ErbB 2 antibody [EPR19547-12] (ab214275) at 1/2000 dilution
+ Human breast cancer lysate at 10 µg

Secondary

Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

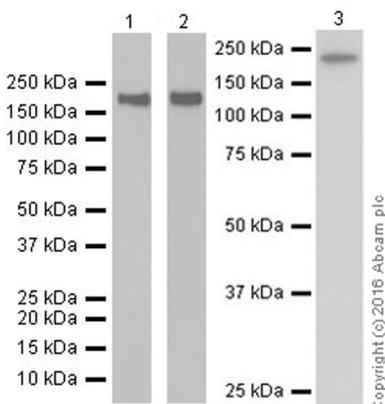
Predicted band size: 137 kDa

Observed band size: 180 kDa [why is the actual band size different from the predicted?](#)

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDm/TBST.

The molecular weight is consist with literature PMID :15741050.



Western blot - Anti-ErbB 2 antibody [EPR19547-12] (ab214275)

Lanes 1-2 : Anti-ErbB 2 antibody [EPR19547-12] (ab214275) at 1/1000 dilution

Lane 3 : Anti-ErbB 2 antibody [EPR19547-12] (ab214275) at 1/5000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2 : A431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 3 : SK-BR-3 (Human mammary gland adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

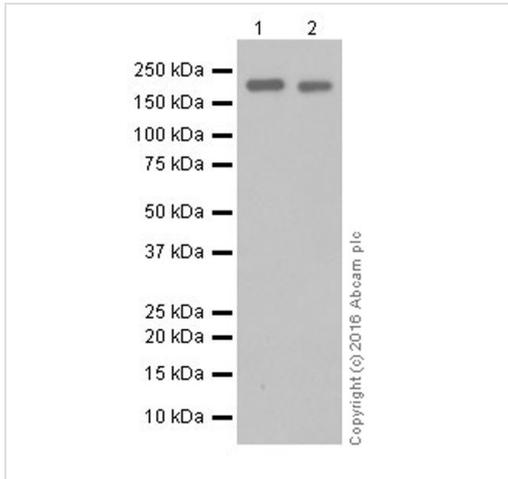
Predicted band size: 137 kDa

Observed band size: 180 kDa [why is the actual band size different from the predicted?](#)

Blocking/Dilution buffer: 5% NFDm/TBST.

Exposure time: Lane 1: 3 minutes; Lane 2: 3 seconds; Lane 3: 10 seconds.

The molecular weight observed is consistent with the literature PMID :15741050.



Western blot - Anti-ErbB 2 antibody [EPR19547-12] (ab214275)

All lanes : Anti-ErbB 2 antibody [EPR19547-12] (ab214275) at 1/1000 dilution

Lane 1 : C6 (Rat glial tumor cell line) whole cell lysate

Lane 2 : PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

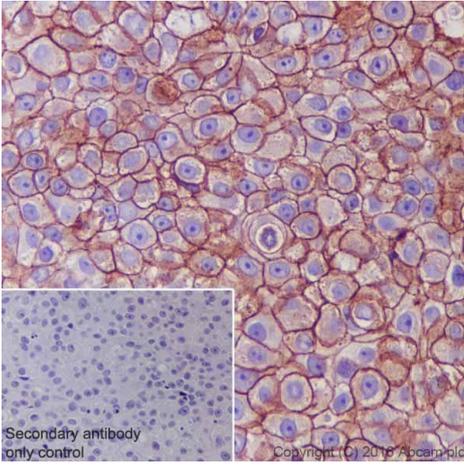
Predicted band size: 137 kDa

Observed band size: 180 kDa [why is the actual band size different from the predicted?](#)

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDm/TBST.

The molecular weight is consist with literature PMID :15741050.



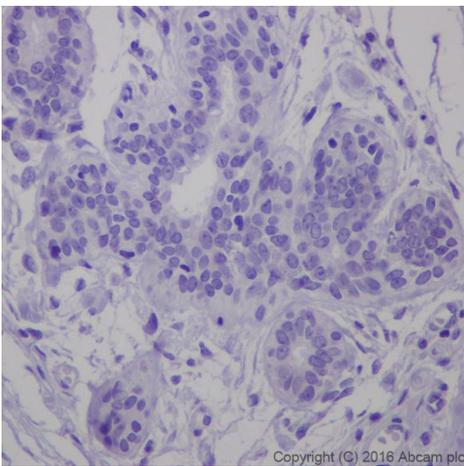
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ErbB 2 antibody [EPR19547-12] (ab214275)

Immunohistochemical analysis of paraffin-embedded Human breast cancer tissue labeling ErbB 2 with ab214275 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

Membrane staining on Human breast cancer is observed [PMID: 18437174].

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ab97051 at 1/500 dilution.

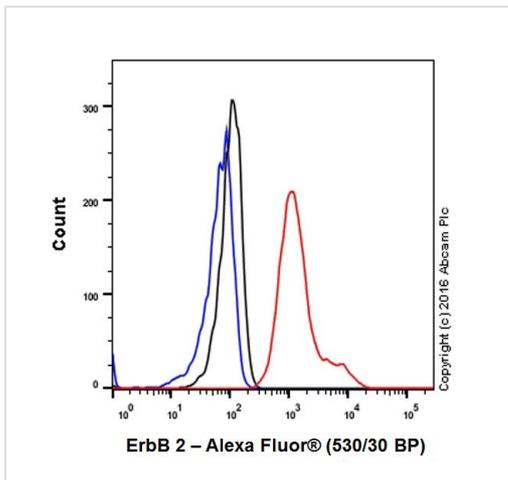


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ErbB 2 antibody [EPR19547-12] (ab214275)

Immunohistochemical analysis of paraffin-embedded Human breast tissue labeling ErbB 2 with ab214275 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

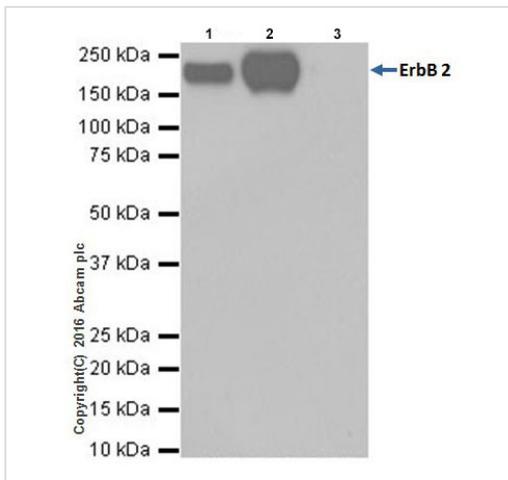
Negative control: No staining on normal Human breast [PMID: 15150568].

Counter stained with Hematoxylin.



Flow Cytometry - Anti-ErbB 2 antibody [EPR19547-12] (ab214275)

Flow cytometric analysis of 4% paraformaldehyde-fixed SK-BR-3 (Human mammary gland adenocarcinoma cell line) cells labeling ErbB 2 with ab214275 at 1/60 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit IgG (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-ErbB 2 antibody [EPR19547-12] (ab214275)

ErbB 2 was immunoprecipitated from 0.35 mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab214275 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab214275 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1000 dilution

Lane 1: HeLa whole cell lysate 10µg (Input).

Lane 2: ab214275 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab214275 in HeLa whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDm/TBST.

Exposure time: 3 seconds.

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