

Product datasheet

Anti-ErbB2 / HER2 antibody [EPR19547-12] ab214275

Recombinant RabMAb

★★★★★ [2 Abreviews](#) [8 References](#) [14 Images](#)

Overview

Product name	Anti-ErbB2 / HER2 antibody [EPR19547-12]
Description	Rabbit monoclonal [EPR19547-12] to ErbB2 / HER2
Host species	Rabbit
Specificity	<p>Only reacts with weakly in mouse 4T1 cells.</p> <p>This antibody could recognize ErbB2 and weak cross reaction with ErbB4.</p> <p>Mouse and rat species are recommended based on WB results, we do not guarantee IHC-P for mouse and rat.</p>
Tested applications	Suitable for: Flow Cyt (Intra), Indirect ELISA, ICC/IF, IP, IHC-P, WB
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human breast cancer tissue. ICC/IF: SK-BR-3 cells. Flow Cyt (intra): SK-BR-3 cells, T-47D cells. IP: HeLa whole cell lysate.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

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	<p>pH: 7.2</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA</p>

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR19547-12
Isotype	IgG

Applications

The Abpromise guarantee 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
Flow Cyt (Intra)		1/60. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
Indirect ELISA		Use at an assay dependent concentration.
ICC/IF	★★★★★ (1)	1/250.
IP		1/30.
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★★★★★ (1)	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.

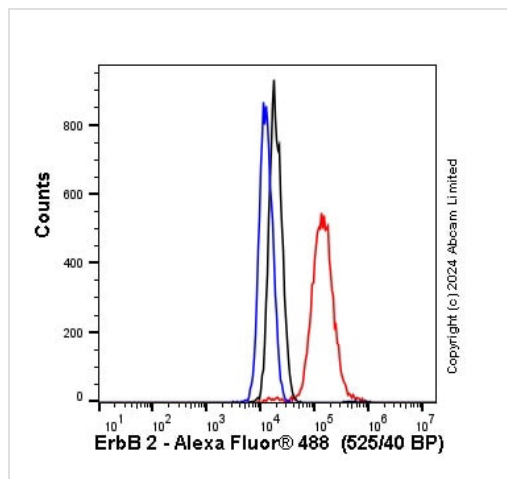
Post-translational modifications

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



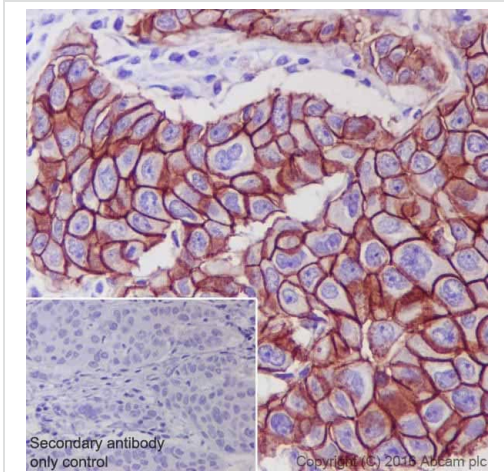
Flow Cytometry (Intracellular) - Anti-ErbB2 / HER2 antibody [EPR19547-12] (ab214275)

Flow cytometry overlay histogram showing T-47D cells stained with ab214275 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab214275) (1×10^6 in 100 μ l at 0.2 μ g/ml (1/2500)) for 30 min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30 min at 22°C. Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW blue laser (488nm) and 525/40 bandpass filter.

This antibody gave a positive signal in T-47D fixed with 80% methanol (5 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ErbB2 / HER2 antibody [EPR19547-12] (ab214275)

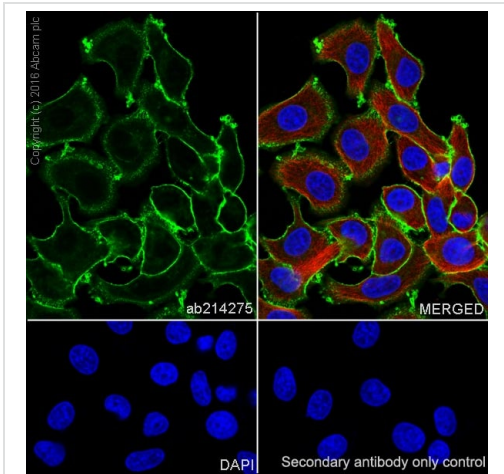
Immunohistochemical analysis of paraffin-embedded Human breast cancer tissue labeling ErbB 2 with ab214275 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/500 dilution.

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.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-ErbB2 / HER2 antibody [EPR19547-12] (ab214275)

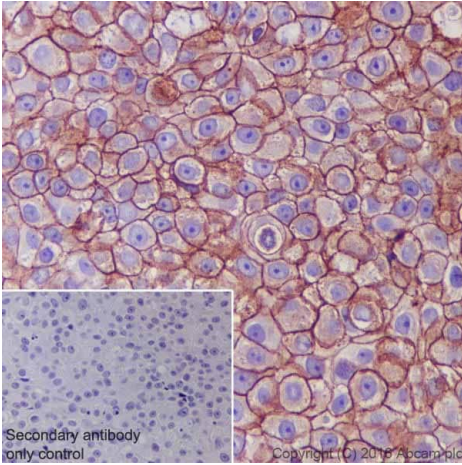
Immunofluorescent analysis of 100% methanol-fixed SK-BR-3 (Human mammary gland adenocarcinoma cell line) cells labeling ErbB 2 with ab214275 at 1/250 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor[®] 488) ([ab150077](#)) secondary antibody at 1/1000 dilution (green).

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-ErbB2 / HER2 antibody [EPR19547-12] (ab214275)

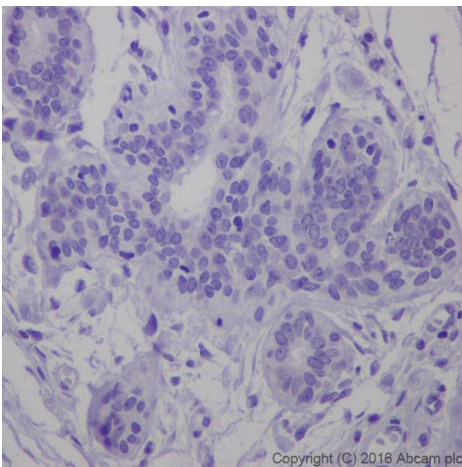
Immunohistochemical analysis of paraffin-embedded Human breast cancer tissue labeling ErbB 2 with ab214275 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



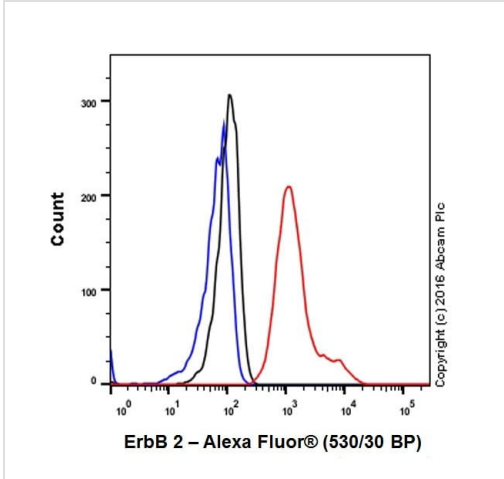
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ErbB2 / HER2 antibody [EPR19547-12] (ab214275)

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].

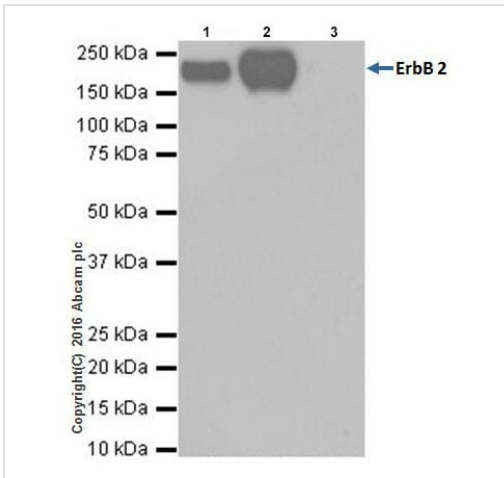
Counter stained with Hematoxylin.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-ErbB2 / HER2 antibody [EPR19547-12] (ab214275)

Intracellular 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-ErbB2 / HER2 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

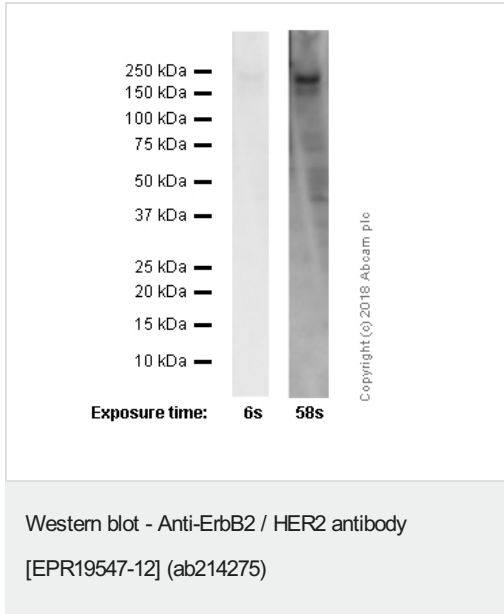
Lane 1: HeLa whole cell lysate 10µg (Input).

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% NFD/MBST.

Exposure time: 3 seconds.



All lanes : Anti-ErbB2 / HER2 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.

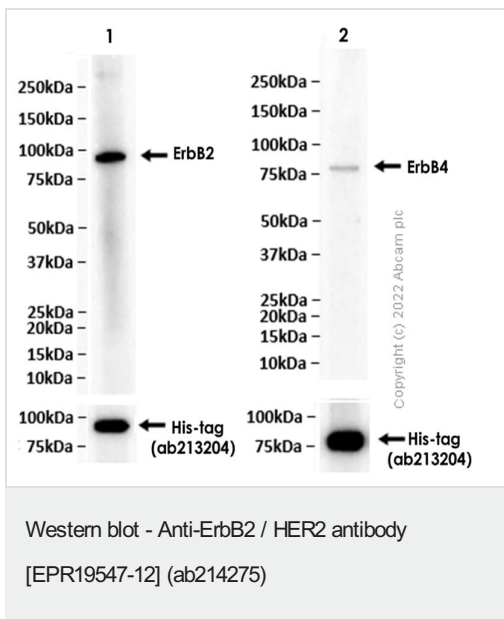
Secondary

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

Blocking and diluting buffer: 5% NFD/MTBST.



All lanes : Anti-ErbB2 / HER2 antibody [EPR19547-12] (ab214275) at 1/1000 dilution

Lane 1 : His-tagged Recombinant Human ErbB2 protein (aa676-1255)

Lane 2 : His-tagged Recombinant Human ErbB4 protein (aa676-1308)

Secondary

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

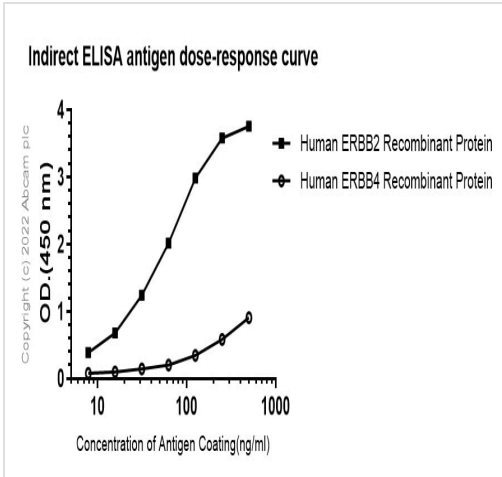
Predicted band size: 137 kDa

Observed band size: 80,90 kDa

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

Exposure time: 180 seconds.

This antibody could recognize ErbB2 and weak cross reaction with ErbB4.



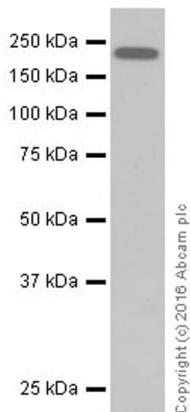
Indirect ELISA - Anti-ErbB2 / HER2 antibody
[EPR19547-12] (ab214275)

ELISA using ab214275 at using 1000 ng/ml an antigen concentration range of 500-0 ng/ml for Human ERBB2 and ERBB4 Recombinant Proteins. Goat Anti-Rabbit IgG (HRL) (1/50000) was used as the secondary antibody. 3,3',5,5'-Tetramethylbenzidine (TMB) used as substrate solution.

Why choose a recombinant antibody?

<p>Research with confidence Consistent and reproducible results</p>	<p>Long-term and scalable supply Recombinant technology</p>
<p>Success from the first experiment Confirmed specificity</p>	<p>Ethical standards compliant Animal-free production</p>

Anti-ErbB2 / HER2 antibody [EPR19547-12]
(ab214275)



Western blot - Anti-ErbB2 / HER2 antibody
[EPR19547-12] (ab214275)

Anti-ErbB2 / HER2 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

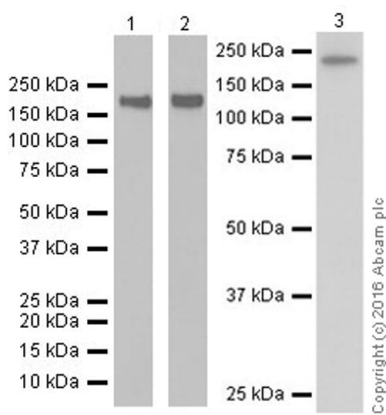
Predicted band size: 137 kDa

Observed band size: 180 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDm/TBST.

The molecular weight is consist with literature PMID :15741050.



Western blot - Anti-ErbB2 / HER2 antibody
[EPR19547-12] (ab214275)

Lanes 1-2 : Anti-ErbB2 / HER2 antibody [EPR19547-12] (ab214275) at 1/1000 dilution

Lane 3 : Anti-ErbB2 / HER2 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

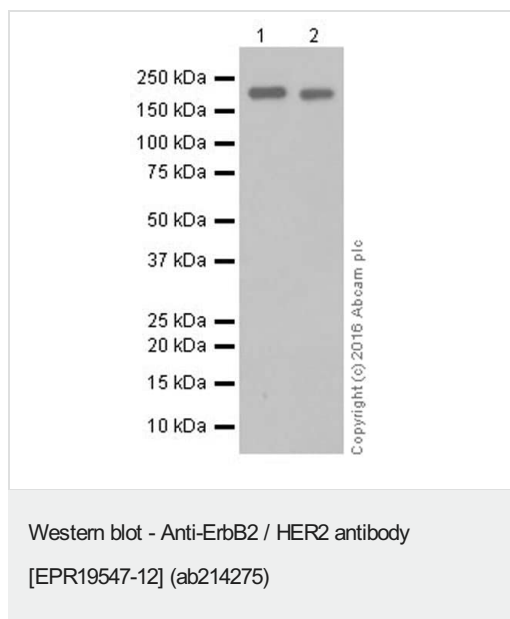
Predicted band size: 137 kDa

Observed band size: 180 kDa

Blocking/Dilution buffer: 5% NFD/MTBST.

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.



All lanes : Anti-ErbB2 / HER2 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

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFD/MTBST.

The molecular weight is consistent with literature PMID :15741050.

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