abcam

Product datasheet

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



重组 RabMAb

★★★★★ 2 Abreviews 8 References 13 图像

概述

产品名称 Anti-ErbB2 / HER2抗体[EPR19547-12]

描述 兔单克隆抗体[EPR19547-12] to ErbB2 / HER2

宿主 Rabbit

特异性 Only reacts with weakly in mouse 4T1 cells.

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

Mouse and rat species are recommended based on WB results, we do not guarantee IHC-P for

mouse and rat.

经测试应用 适用于: Flow Cyt (Intra), Indirect ELISA, ICC/IF, IP, IHC-P, WB

种属反应性 与反应: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

阳性对照 IHC-P: Human breast cancer tissue. ICC/IF: SK-BR-3 cells. Flow Cyt (intra): SK-BR-3 cells. IP:

HeLa whole cell lysate.

常规说明 This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

性能

形式 Liquid

存放说明 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.

存储溶液 pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

纯**度** Protein A purified

克隆 单克隆

克隆编号 EPR19547-12

同种型 IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab214275于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/60. ab172730 - Rabbit monoclonal lgG, 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).

靶标

功能

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.

组织特异性

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

疾病相关 Hereditary diffuse gastric cancer

Glioma

Ovarian cancer Lung cancer

2

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.

序列相似性 Belongs to the protein kinase superfamily. Tyr protein kinase family. EGF receptor subfamily.

Contains 1 protein kinase domain.

翻译后修饰 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

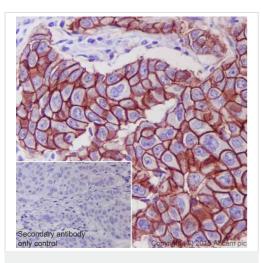
(PubMed:27134172).

细胞定位 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.

图片



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

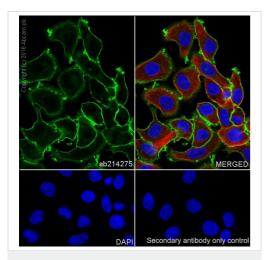
Immunohistochemical analysis of paraffin-embedded Human breast cancer tissue labeling ErbB 2 with ab214275 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

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 <u>ab97051</u> 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)

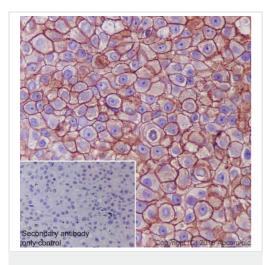
Immunofluorescent analysis of 100% methanol-fixed SK-BR-3 (Human mammary gland adenocarcinoma cell line) cells labeling ErbB 2 with ab214275 at 1/250 dilution, followed by Goat Anti-Rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

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

The nuclear counterstain is DAPI (blue).

Tubulin is detected with <u>ab195889</u> 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 lgG (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution.



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

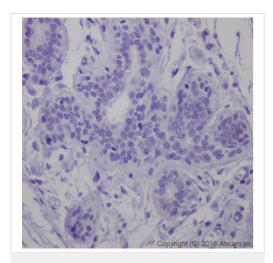
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Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



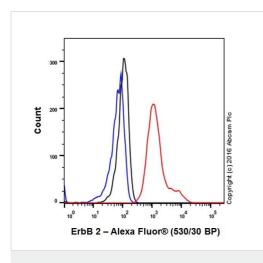
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ErbB2 / HER2 antibody [EPR19547-12] (ab214275)

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].

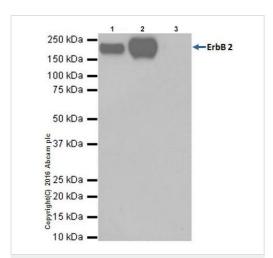
Counter stained with Hematoxylin.

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 RabbitlgG,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) (<u>ab131366</u>), 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 lgG ($\underline{ab172730}$) instead of ab214275 in HeLa whole cell lysate.

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

250 kDa —
150 kDa —
100 kDa —
75 kDa —
50 kDa —
37 kDa —
25 kDa —
20 kDa —
15 kDa —
10 kDa —
10 kDa —

10 kDa —

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

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) (<u>ab97051</u>) 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% NFDM/TBST.

2 1 250kDa -250kDa 150kDa -150kDa -100kDa -100kDa -ErbB2 - ErbB4 75kDa -75kDa -50kDa -50kDa -37kDa -37kDa -25kDa -20kDa -25kDa -20kDa -15kDa -15kDa -10kDa -10kDa -100kDa 100kDa His-taa ■ His-tag 75kDa -(ab213204) 75kDa -(ab213204)

Western blot - Anti-ErbB2 / HER2 antibody [EPR19547-12] (ab214275) **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 lgG H&L (HRP) (ab97051) at 1/20000 dilution

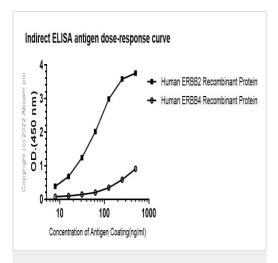
Predicted band size: 137 kDa **Observed band size:** 80,90 kDa

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 lgG (HRL) (1/50000) was used as the secondary antibody. 3,3',5,5'-Tetramethylbenzidine (TMB) used as substrate solution.



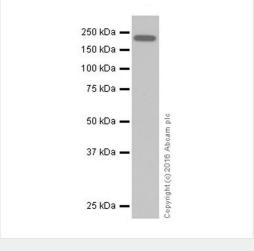


specificity

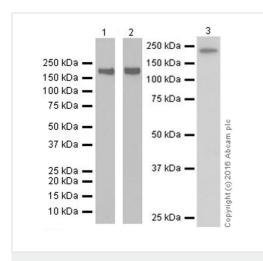


production

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



Western blot - 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 lgG Peroxidase Conjugate, specific to the non-reduced form of lgG 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.

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 lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 137 kDa **Observed band size:** 180 kDa

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.

1 2

250 kDa —

150 kDa —

100 kDa —

75 kDa —

50 kDa —

37 kDa —

25 kDa —

20 kDa —

15 kDa —

10 kDa —

10 kDa —

10 kDa —

10 kDa —

[EPR19547-12] (ab214275)

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 lgG 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% NFDM/TBST.

The molecular weight is consist with literature PMID:15741050.

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