abcam

Product datasheet

Anti-ErbB2 / HER2 antibody [CAL27] ab237715

敲除验证 重组 RabMAb

3 References 11 图像

概述

产品名称	Anti-ErbB2 / HER2抗体[CAL27]
描述	兔单克隆抗体[CAL27] to ErbB2 / HER2
宿主	Rabbit
经测试应用	**适用于:** WB, ICC/IF, IP, Flow Cyt (Intra), IHC-P
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	WB: HeLa, 4T1, C6, Wild-type HCT 116, Wild-type A549 and SK-BR-3 whole cell lysates. IHC-P: Human breast carcinoma tissue. ICC/IF: SK-BR-3 cells. Flow Cyt (intra): SK-BR-3 cells. IP: HeLa whole cell lysate.

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: 7.50 Preservative: 0.05% Sodium azide Constituent: PBS
纯度	Protein A purified
纯化说明	Purity >99%.
克隆	单克隆
克隆编号	CAL27
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab237715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
WB		1/1000. Detects a band of approximately 180 kDa (predicted molecular weight: 137 kDa).
ICC/IF		1/500.
IP		1/13.
Flow Cyt (Intra)		1/500.
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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
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.

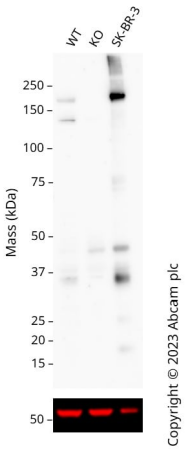
翻译后修饰

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



Western blot - Anti-ErbB2 / HER2 antibody [CAL27] (ab237715)

All lanes : Anti-ErbB2 / HER2 antibody [CAL27] (ab237715) at 1/500 dilution

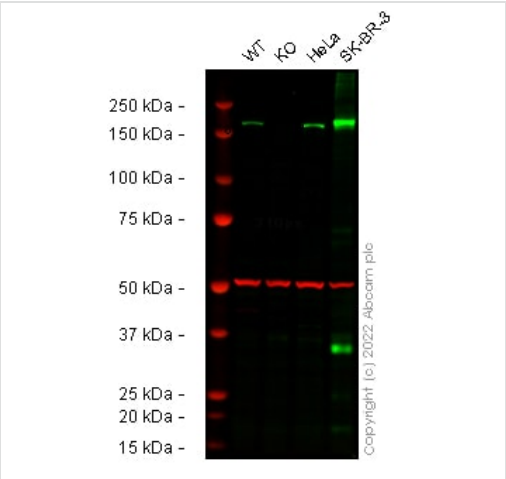
Lane 1 : Wild-type MCF7 cell lysate at 32 µg
Lane 2 : ERBB2 knockout MCF7 cell lysate at 32 µg
Lane 3 : SK-BR-3 cell lysate at 16 µg

Performed under reducing conditions.

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

Anti-ErbB2 / HER2 antibody [CAL27] staining at 1/500 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab237715 was shown to bind specifically to ErbB2 / HER2. A band was observed at 180 kDa in wild-type MCF7 cell lysates with no signal observed at this size in ERBB2 knockout cell line **ab286260** (knockout cell lysate AB300208).

To generate this image, wild-type and ERBB2 knockout MCF7 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were HRP conjugated Goat anti-Rabbit (H+L) and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-ErbB2 / HER2 antibody [CAL27] (ab237715)

All lanes : Anti-ErbB2 / HER2 antibody [CAL27] (ab237715) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : ERBB2 knockout A549 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : SK-BR-3 cell lysate

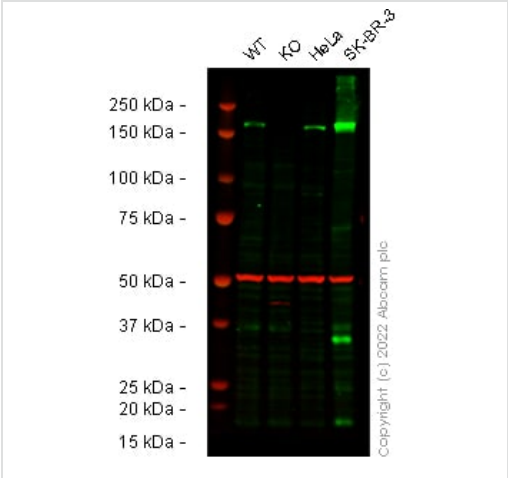
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

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

False colour image of Western blot: Anti-ErbB2 / HER2 antibody [CAL27] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab237715 was shown to bind specifically to ErbB2 / HER2. A band was observed at 180 kDa in wild-type A549 cell lysates with no signal observed at this size in ERBB2 knockout cell line. To generate this image, wild-type and ERBB2 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-ErbB2 / HER2 antibody [CAL27] (ab237715)

All lanes : Anti-ErbB2 / HER2 antibody [CAL27] (ab237715) at 1/1000 dilution

Lane 1 : Wild-type HCT 116 cell lysate
Lane 2 : ERBB2 knockout HCT 116 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : SK-BR-3 cell lysate

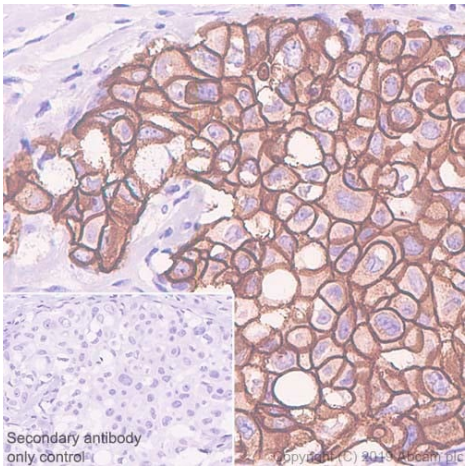
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

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

False colour image of Western blot: Anti-ErbB2 / HER2 antibody [CAL27] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab237715 was shown to bind specifically to ErbB2 / HER2. A band was observed at 180 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in ERBB2 knockout cell line. To generate this image, wild-type and ERBB2 knockout HCT 116 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ErbB2 / HER2 antibody [CAL27] (ab237715)

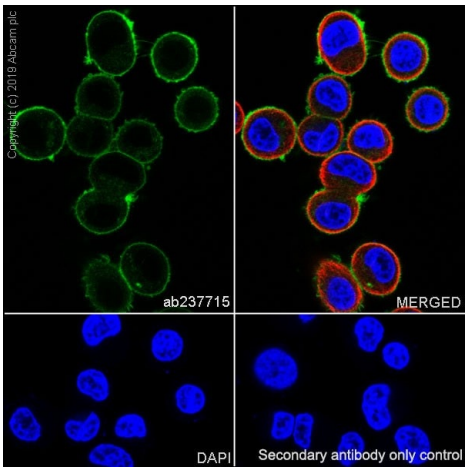
Immunohistochemical analysis of human breast carcinoma tissue labeling ErbB2 / HER2 with ab237715 at 1/2000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on the human breast carcinoma is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

The section was incubated with ab237715 for 10 mins at room temperature.

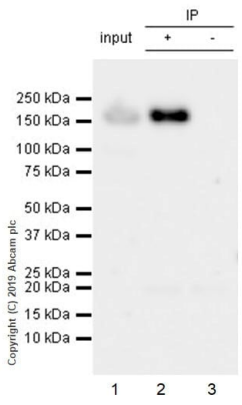
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunocytochemistry/ Immunofluorescence - Anti-ErbB2 / HER2 antibody [CAL27] (ab237715)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilised SK-BR-3 (human mammary gland adenocarcinoma cell line) cells labeling ERbB 2 with ab237715 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous staining in SK-BR-3 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.

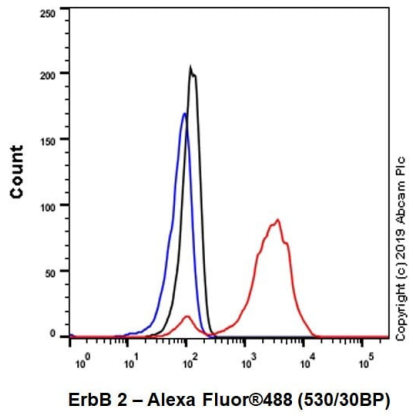


Immunoprecipitation - Anti-ErbB2 / HER2 antibody [CAL27] (ab237715)

ERbB 2 was immunoprecipitated from 0.35 mg of HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab237715 at 130 dilution. Western blot was performed from the immunoprecipitate using ab237715 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used as secondary antibody at 1/5000 dilution.

Lane 1: HeLa whole cell lysate 10 µg (Input).
Lane 2: ab237715 IP in HeLa whole lysate.
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab237715 in HeLa whole cell lysate.

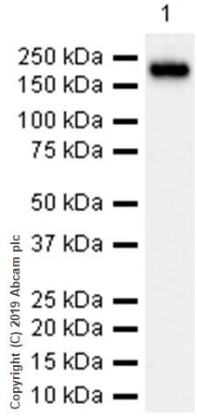
Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 15 seconds.



Flow Cytometry (Intracellular) - Anti-ErbB2 / HER2 antibody [CAL27] (ab237715)

Intracellular flow cytometric analysis of4% paraformaldehyde-fixed, 90% methonao-permeabilized SH-BR-3 (human mammary gland adenocarcinoma cell line) cells labeling ErbB2 / HER2 with ab237715 at 1/500 dilution (red) compared with Recombinant 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 H&L (Alexa Fluor® 488) (**ab150077**), at 1/2000 dilution was used as the secondary antibody.



Western blot - Anti-ErbB2 / HER2 antibody [CAL27] (ab237715)

Anti-ErbB2 / HER2 antibody [CAL27] (ab237715) at 1/1000 dilution + HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg

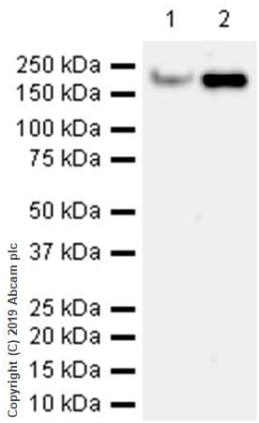
Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Exposure time: 3 minutes

Blocking and dilution buffer: 5% NFDM/TBST.

This blot was developed using a higher sensitivity ECL substrate.



Western blot - Anti-ErbB2 / HER2 antibody [CAL27] (ab237715)

All lanes : Anti-ErbB2 / HER2 antibody [CAL27] (ab237715) at 1/1000 dilution

Lane 1 : 4T1 (mouse mammary gland carcinoma cell line) whole cell lysate
Lane 2 : C6 (rat glial tumor glial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

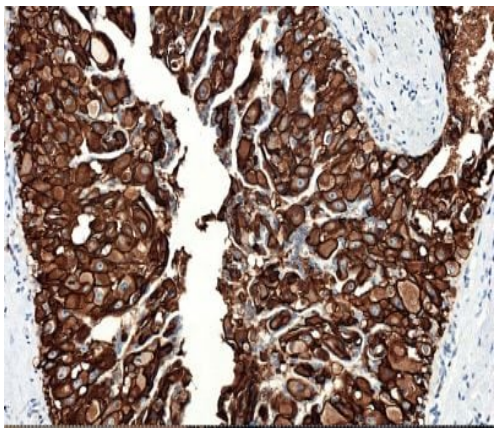
Secondary
All lanes : VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at 1/5000 dilution

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

Exposure time: 3 minutes

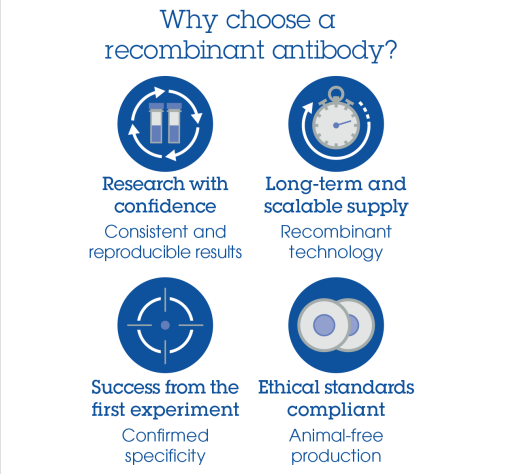
Blocking and dilution buffer: 5% NFDM/TBST.

This blot was developed using a higher sensitivity ECL substrate.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ErbB2 / HER2 antibody [CAL27] (ab237715)

Formalin-fixed, paraffin-embedded human breast carcinoma tissue stained for ErbB2 / HER2 using ab237715 at 0.3 µg/ml in immunohistochemical analysis.



Anti-ErbB2 / HER2 antibody [CAL27] (ab237715)

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