abcam

Product datasheet

Anti-ErbB2 / HER2 antibody [CAL27] - BSA and Azide free ab251602

KO VALIDATED Recombinant RabMAb

9 Images

Overview

Product name	Anti-ErbB2 / HER2 antibody [CAL27] - BSA and Azide free
Description	Rabbit monoclonal [CAL27] to ErbB2 / HER2 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, WB, ICC/IF, IP, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	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.
General notes	ab251602 is the carrier-free version of **ab237715**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2

	Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Purification notes	Purity is greater than 99%.
Clonality	Monoclonal
Clone number	CAL27
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab251602 in the following tested applications.

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

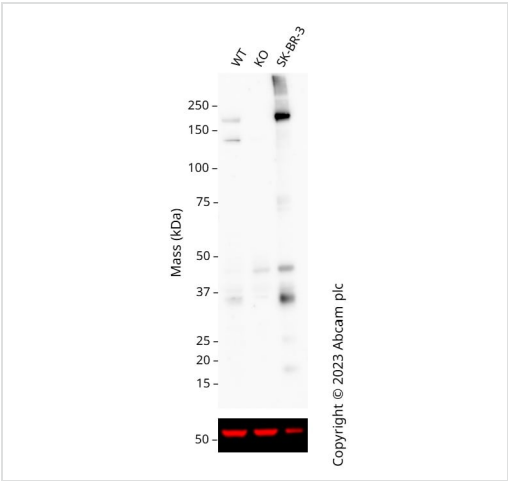
Application	Abreviews	Notes
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 180 kDa (predicted molecular weight: 137 kDa).
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.

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



Western blot - Anti-ErbB2 / HER2 antibody [CAL27] - BSA and Azide free (ab251602)

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.

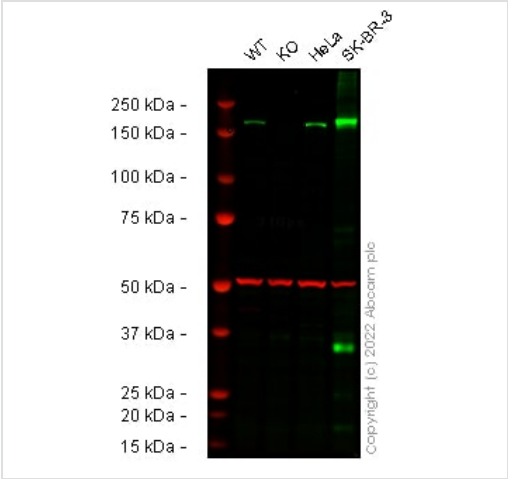
Predicted band size: 137 kDa
Observed band size: 180 kDa

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (**ab237715**).

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] - BSA and Azide free (ab251602)

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

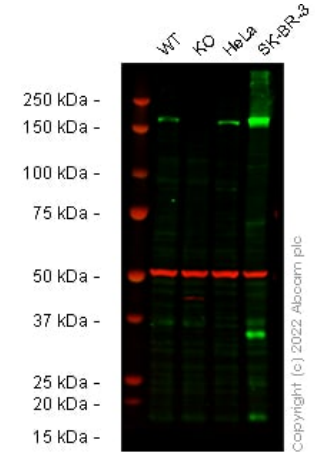
Performed under reducing conditions.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (**ab237715**).



Western blot - Anti-ErbB2 / HER2 antibody [CAL27] - BSA and Azide free (ab251602)

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

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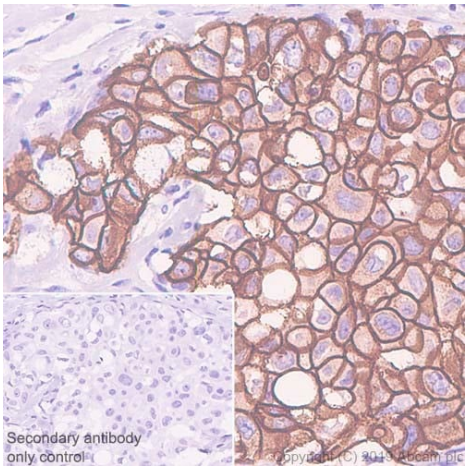
False colour image of Western blot: Anti-ErbB2 / HER2 antibody [CAL27] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab237715** was shown to bind specifically to ErbB2 / HER2. A 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide

(**ab237715**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ErbB2 / HER2 antibody [CAL27] - BSA and Azide free (ab251602)

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.

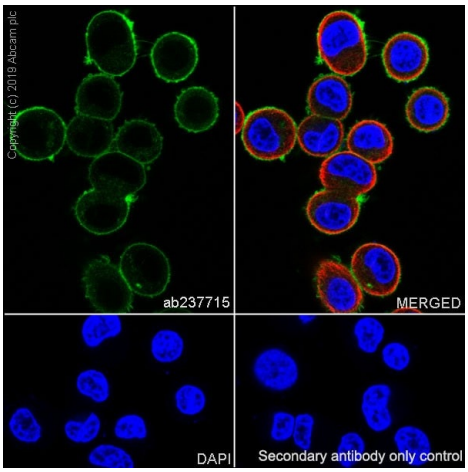
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (**ab237715**).

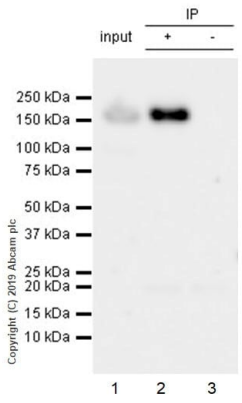


Immunocytochemistry/ Immunofluorescence - Anti-ErbB2 / HER2 antibody [CAL27] - BSA and Azide free (ab251602)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilised SK-BR-3 (human mammary gland adenocarcinoma cell line) cells labeling ErbB2 / HER2 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (**ab237715**).



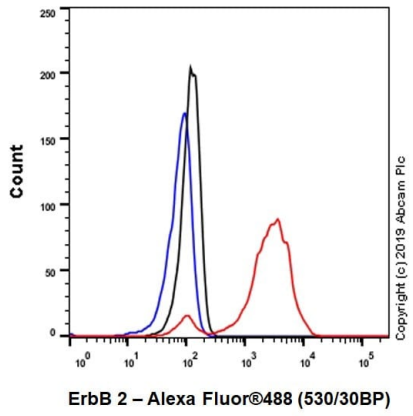
Immunoprecipitation - Anti-ErbB2 / HER2 antibody [CAL27] - BSA and Azide free (ab251602)

ErbB2 / HER2 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (**ab237715**).

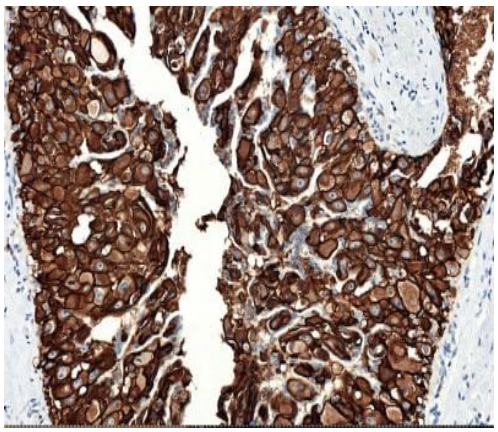


Flow Cytometry (Intracellular) - Anti-ErbB2 / HER2 antibody [CAL27] - BSA and Azide free (ab251602)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (**ab237715**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ErbB2 / HER2 antibody [CAL27] - BSA and Azide free (ab251602)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS and sodium azide (**ab237715**).

Anti-ErbB2 / HER2 antibody [CAL27] - BSA and Azide free (ab251602)

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