abcam

Product datasheet

Human ErbB2 / HER2 knockout MCF7 cell line ab286260

3 Images

Overview

Product name	Human ErbB2 / HER2 knockout MCF7 cell line
Parental Cell Line	MCF7
Organism	Human
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS), Western Blot (WB)
Tested applications	**Suitable for:** Next Generation Sequencing
Biosafety level	1
General notes	Although we aim to provide customers with a homozygous clone, feasibility will be dependent on the biology of the protein. Should only heterozygous edits be achieved, you will be notified of the outcome and be asked to confirm whether the cell line is acceptable. All clones will be accompanied with DNA sequencing data, and the mutation description. **Recommended control**: Human wild-type MCF7 cell line (**ab288560**). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1. **Cryopreservation cell medium**: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium**: MEM + 10% FBS + 0.01 mg/mL bovine insulin **Initial handling guidelines**: Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution. 3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of 5-7x10^4cells/cm2. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO2. Cultures should be monitored daily. **Subculture guidelines**: All seeding densities should be based on cell counts gained by established methods.

A guide seeding density of 5-7x10^4 cells/cm2 is recommended.
A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.
Cells should be passaged when they have achieved 80-90% confluence.
This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.

We will provide viable cells that proliferate on revival.

Properties

Number of cells	1000000 cells/vial, 1 mL
Viability	~80
Adherent /Suspension	Adherent
Tissue	Breast
Cell type	epithelial
Disease	Adenocarcinoma
Gender	Female
Sequencing result	143 bp deletion after the Gln90 of the WT protein.
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

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.

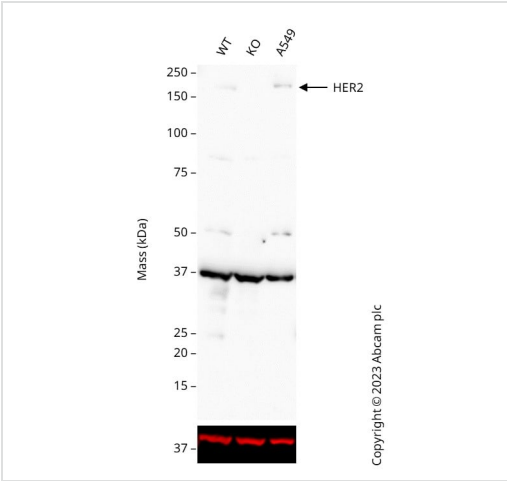
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab286260 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
Next Generation Sequencing		Use at an assay dependent concentration.

Images



Western blot - Human ErbB2 / HER2 knockout MCF7 cell line (ab286260)

All lanes : Anti-ErbB2 / HER2 antibody [EP1045Y] (**ab134182**) at 1/500 dilution

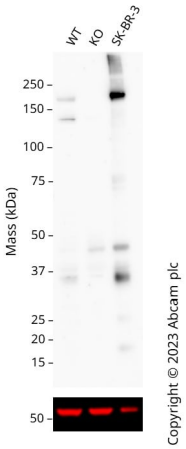
Lane 1 : Wild-type MCF7 cell lysate at 32 µg
Lane 2 : ERBB2 knockout MCF7 cell lysate at 32 µg
Lane 3 : A549 cell lysate at 16 µg

Performed under reducing conditions.

Observed band size: 180 kDa

Western blot: Anti-ErbB2 / HER2 antibody [EP1045Y] staining at 1/500 dilution, shown in black; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab134182** 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 and washed again four times. Secondary antibodies used were HRP conjugated Goat anti-Rabbit (H+L) and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution. This blot was developed with an ultra high-sensitivity ECL substrate kit and imaged with 20 minutes exposure time.



Western blot - Human ErbB2 / HER2 knockout MCF7 cell line (ab286260)

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

Secondary
All lanes : HRP conjugated Goat anti-Rabbit (H+L) and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

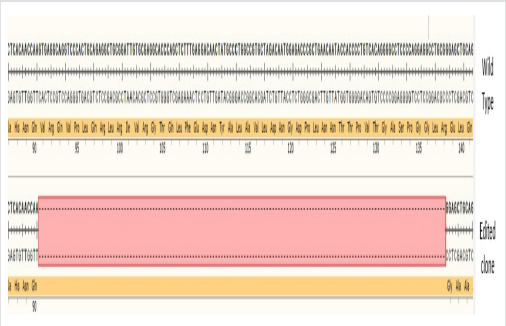
Performed under reducing conditions.

Observed band size: 180 kDa

Western blot: 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.



Next Generation Sequencing - Human ErbB2 / HER2 knockout MCF7 cell line (ab286260)

143 bp deletion after the Gln90 of the WT protein.

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