

# Human EGFR knockout HCT116 cell line ab281597

2 Images

### Overview

Product name	Human EGFR knockout HCT116 cell line
Parental Cell Line	HCT116
Organism	Human
Passage number	<20
Knockout validation	Sanger Sequencing
Biosafety level	1
General notes	<p><b>Recommended control:</b> Human wild-type HCT116 cell line (<a href="#">ab273730</a>). 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.</p> <p><b>Cryopreservation cell medium:</b> Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p><b>Culture medium:</b> McCoY5a + 10% FBS</p> <p><b>Initial handling guidelines:</b> 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.</p> <ol style="list-style-type: none"> <li>1. Thaw the vial in 37°C water bath for approximately 1-2 minutes.</li> <li>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.</li> <li>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 <math>2 \times 10^4</math> cells/cm<sup>2</sup>. Seeding density is given as a guide only and should be scaled to align with individual lab schedules.</li> <li>4. Incubate the culture at 37°C incubator with 5% CO<sub>2</sub>. Cultures should be monitored daily.</li> </ol> <p><b>Subculture guidelines:</b></p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of <math>2 \times 10^4</math> cells/cm<sup>2</sup> is recommended.</p> <p>A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.</p> <p>Cells should be passaged when they have achieved 80-90% confluence.</p> <p>This product is subject to limited use licenses from The Broad Institute and ERS Genomics</p>

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

<b>Number of cells</b>	1 x 10 <sup>6</sup> cells/vial, 1 mL
<b>Adherent /Suspension</b>	Adherent
<b>Tissue</b>	Colon
<b>Cell type</b>	epithelial
<b>Disease</b>	Carcinoma
<b>Gender</b>	Male
<b>Mycoplasma free</b>	Yes
<b>Storage instructions</b>	Shipped on Dry Ice. Store in liquid nitrogen.
<b>Storage buffer</b>	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

## Target

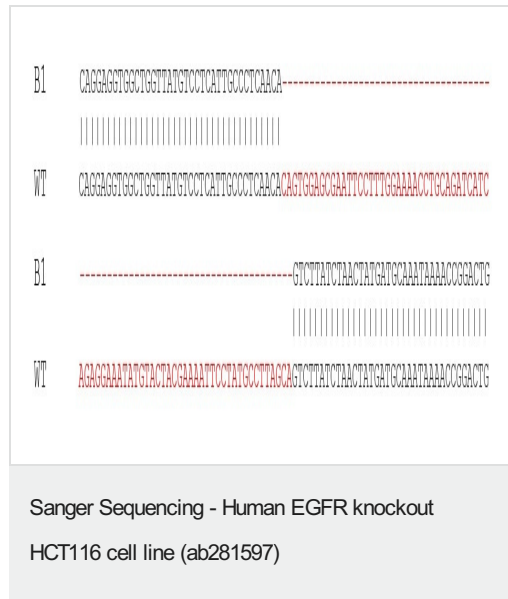
<b>Function</b>	<p>Receptor tyrosine kinase binding ligands of the EGF family and activating several signaling cascades to convert extracellular cues into appropriate cellular responses. Known ligands include EGF, TGFA/TGF-<math>\alpha</math>, amphiregulin, epigen/EPGN, BTC/betacellulin, epiregulin/EREG and HBEGF/heparin-binding EGF. Ligand binding triggers receptor homo- and/or heterodimerization and autophosphorylation on key cytoplasmic residues. The phosphorylated receptor recruits adapter proteins like GRB2 which in turn activates complex downstream signaling cascades. Activates at least 4 major downstream signaling cascades including the RAS-RAF-MEK-ERK, PI3 kinase-AKT, PLCgamma-PKC and STATs modules. May also activate the NF-kappa-B signaling cascade. Also directly phosphorylates other proteins like RGS16, activating its GTPase activity and probably coupling the EGF receptor signaling to the G protein-coupled receptor signaling. Also phosphorylates MUC1 and increases its interaction with SRC and CTNNB1/beta-catenin.</p> <p>Isoform 2 may act as an antagonist of EGF action.</p>
<b>Tissue specificity</b>	Ubiquitously expressed. Isoform 2 is also expressed in ovarian cancers.
<b>Involvement in disease</b>	Lung cancer Inflammatory skin and bowel disease, neonatal, 2
<b>Sequence similarities</b>	Belongs to the protein kinase superfamily. Tyr protein kinase family. EGF receptor subfamily. Contains 1 protein kinase domain.
<b>Post-translational modifications</b>	<p>Phosphorylation at Ser-695 is partial and occurs only if Thr-693 is phosphorylated. Phosphorylation at Thr-678 and Thr-693 by PRKD1 inhibits EGF-induced MAPK8/JNK1 activation. Dephosphorylation by PTPRJ prevents endocytosis and stabilizes the receptor at the plasma membrane. Autophosphorylation at Tyr-1197 is stimulated by methylation at Arg-1199 and enhances interaction with PTPN6. Autophosphorylation at Tyr-1092 and/or Tyr-1110 recruits STAT3. Dephosphorylated by PTPN1 and PTPN2.</p> <p>Monoubiquitinated and polyubiquitinated upon EGF stimulation; which does not affect tyrosine kinase activity or signaling capacity but may play a role in lysosomal targeting. Polyubiquitin linkage is mainly through 'Lys-63', but linkage through 'Lys-48', 'Lys-11' and 'Lys-29' also occurs.</p>

Deubiquitination by OTUD7B prevents degradation. Ubiquitinated by RNF115 and RNF126. Methylated. Methylation at Arg-1199 by PRMT5 stimulates phosphorylation at Tyr-1197.

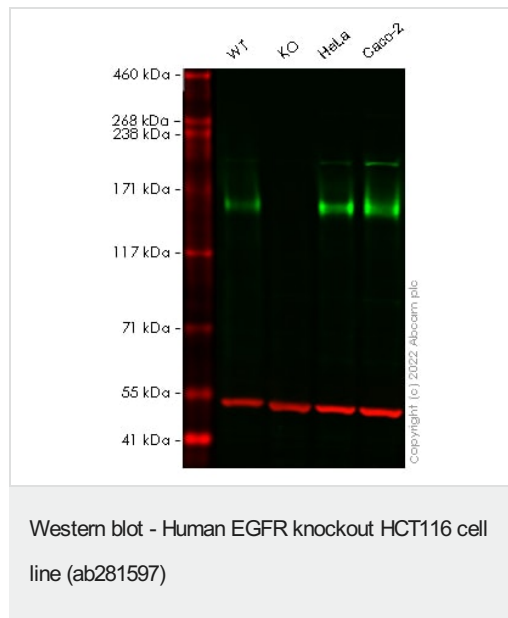
## Cellular localization

Secreted and Cell membrane. Endoplasmic reticulum membrane. Golgi apparatus membrane. Nucleus membrane. Endosome. Endosome membrane. Nucleus. In response to EGF, translocated from the cell membrane to the nucleus via Golgi and ER. Endocytosed upon activation by ligand. Colocalized with GPER1 in the nucleus of estrogen agonist-induced cancer-associated fibroblasts (CAF).

## Images



B1 – 77 bp deletion (Main clone)



**All lanes** : Anti-EGFR antibody [E235] ([ab32077](#)) at 1/1000 dilution

**Lane 1** : Wild-type HCT 116 cell lysate

**Lane 2** : EGFR knockout HCT 116 cell lysate

**Lane 3** : HeLa cell lysate

**Lane 4** : Caco-2 cell lysate

Lysates/proteins at 20 µg per lane.

Developed using the ECL technique.

Performed under reducing conditions.

**Observed band size:** 180 kDa

False colour image of Western blot: Anti-EGFR antibody [E235] 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, [ab32077](#) was shown to bind specifically to EGFR. A band was observed at 180 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in Egfr knockout cell line ab281597 (knockout cell lysate [ab282949](#)). To generate this image, wild-type and Egfr 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 (IRDye® 800CW) preabsorbed ([ab216773](#)) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ([ab216776](#)) at 1/20000 dilution.

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