

Human EGFR knockout HeLa cell line ab255385

5 Images

Overview

Product name	Human EGFR knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon 1 and 1 bp insertion in exon 1
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	Suitable for: WB
Biosafety level	2
General notes	<p>Recommended control: Human wild-type HeLa cell line (ab255928). 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>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: DMEM (High Glucose) + 10% FBS</p> <p>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.</p> <ol style="list-style-type: none"> 1. 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 2×10^4 cells/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. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of 2×10^4 cells/cm² is recommended.</p> <p>A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.</p>

Cells should be passaged when they have achieved 80-90% confluence.

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Properties

Number of cells	1 x 10 ⁶ cells/vial, 1 mL
Viability	~80%
Adherent /Suspension	Adherent
Tissue	Cervix
Cell type	epithelial
Disease	Adenocarcinoma
Gender	Female
STR Analysis	Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8,12 CSF1PO: 9, 10
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	<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-alpha, 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>
Tissue specificity	Ubiquitously expressed. Isoform 2 is also expressed in ovarian cancers.
Involvement in disease	Lung cancer Inflammatory skin and bowel disease, neonatal, 2
Sequence similarities	Belongs to the protein kinase superfamily. Tyr protein kinase family. EGF receptor subfamily. Contains 1 protein kinase domain.
Post-translational modifications	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.

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

Cellular localization

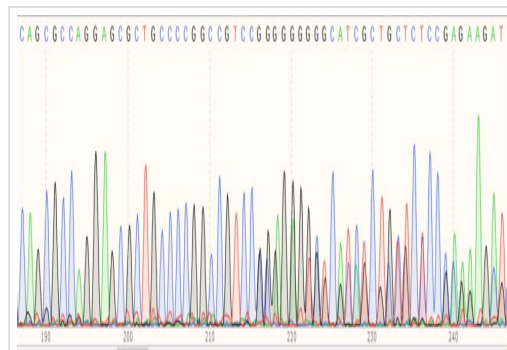
Secreted and Cell membrane. Endoplasmic reticulum membrane. Golgi apparatus membrane. Nucleus membrane. Endosome. Endosome membrane. Nucleus. In response to EGF, translocated from the cell membrane to the nucleus via Golgi and ER. Endocytosed upon activation by ligand. Colocalized with GPER1 in the nucleus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

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab255385 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

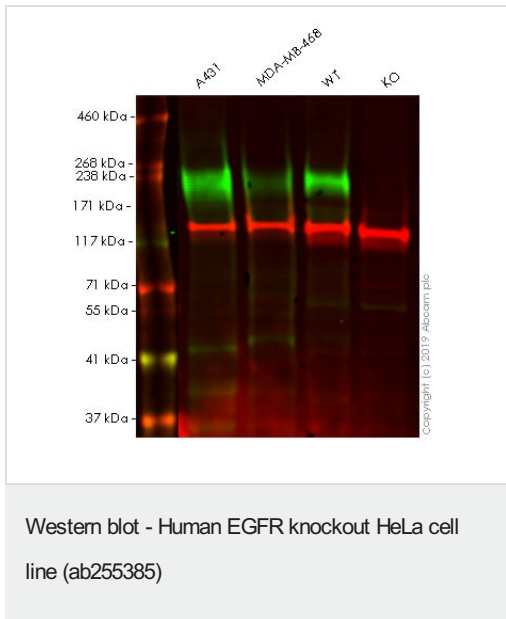
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 134 kDa.

Images



Sequencing chromatogram displaying sequence edit in exon 1

Sanger Sequencing - Human EGFR knockout HeLa cell line (ab255385)



All lanes : Anti-EGFR antibody [EP38Y] (**ab52894**) at 1/1000 dilution

Lane 1 : A431 cell lysate

Lane 2 : MDA-MB-468 cell lysate

Lane 3 : Wild-type HeLa cell lysate

Lane 4 : EGFR knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/20000 dilution

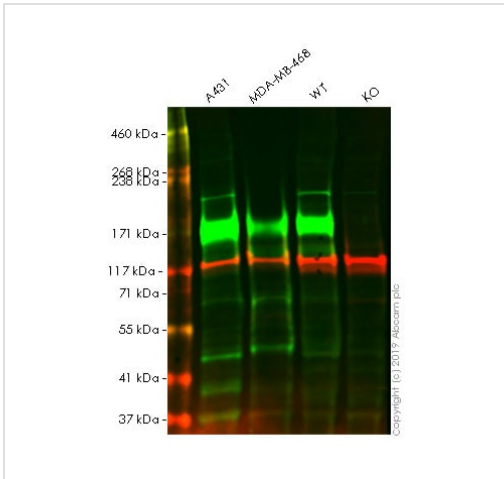
Performed under reducing conditions.

Predicted band size: 134 kDa

Additional bands at: 124 kDa (possible Loading Control)

Lanes 1 - 4: Merged signal (red and green). Green - **ab52894** observed at 175 kDa. Red - loading control, **ab130007** observed at 125 kDa.

ab52894 was shown to react with EGFR in wild-type HeLa. Loss of signal was observed when knockout cell line ab255385 (knockout cell lysate **ab263845**) was used. Wild-type and EGFR knockout samples were subjected to SDS-PAGE. **ab52894** and Anti-Vinculin antibody [VIN-54] (**ab130007**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human EGFR knockout HeLa cell line (ab255385)

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

Lane 1 : A431 cell lysate

Lane 2 : MDA-MB-468 cell lysate

Lane 3 : Wild type HeLa cell lysate

Lane 4 : EGFR knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/20000 dilution

Predicted band size: 134 kDa

Observed band size: 124 kDa

Lanes 1 -4: Merged signal (red and green). Green - **ab32077** observed at 175 kDa. Red - loading control, **ab130007** observed at 125 kDa.

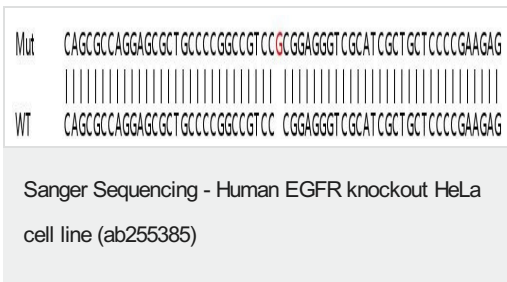
ab32077 was shown to react with EGFR in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab255385 (knockout cell lysate **ab263845**) was used. Wild-type and EGFR knockout samples were subjected to SDS-PAGE. **ab32077** and Anti-Vinculin antibody [VIN-54] (**ab130007**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

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Mut  CAGCGCCAGGAGCGCTGCCCGGCCGTCC-GGAGGGTCGCATCGCTGCTCCCCGAAGAGC
      |||
WT   CAGCGCCAGGAGCGCTGCCCGGCCGTCCCGAGGGTCGCATCGCTGCTCCCCGAAGAGC
  
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Sanger Sequencing - Human EGFR knockout HeLa cell line (ab255385)

Allele-1: 1 bp deletion in exon 1.



Allele-2: 1 bp insertion in exon 1.

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