abcam

Product datasheet

Human NEK6 knockout HeLa cell line ab265910

画像数 5

製品の概要

製品名	Human NEK6 knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 3 and 304 bp deletion in exon 3 and Insertion of the selection cassette in exon 3
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
アプリケーション	**適用あり:** WB
Biosafety level	2
特記事項	**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. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM (High Glucose) + 10% FBS **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. 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 $2x10^4$ cells/cm^2. 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% CO_2. 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 $2x10^4$ cells/cm^2 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.
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We will provide viable cells that proliferate on revival.

製品の特性

Number of cells	1 x 10^6 cells/vial, 1 mL
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
保存方法	Shipped on Dry Ice. Store in liquid nitrogen.
バッファー	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

ターゲット情報

機能	Activated during M phase. Required for chromosome segregation at metaphase-anaphase transition and therefore for mitotic progression. Inhibition of activity results in apoptosis. Phosphorylates KIF11 to promote mitotic spindle formation.
組織特異性	Ubiquitous, with highest expression in heart and skeletal muscle.
配列類似性	Belongs to the protein kinase superfamily. NEK Ser/Thr protein kinase family. NIMA subfamily. Contains 1 protein kinase domain.
翻訳後修飾	Autophosphorylated.
細胞内局在	Cytoplasm. Nucleus.

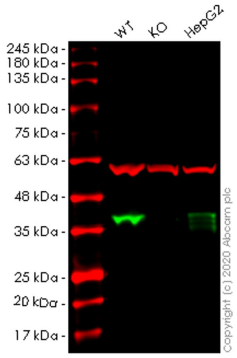
アプリケーション

The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab265910の使用に適用されます**

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 36 kDa.

画像



Western blot - Human NEK6 knockout HeLa cell line (ab265910)

All lanes : Anti-VEGF Receptor 3 antibody (**ab10977**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : NEK6 knockout HeLa cell lysate
Lane 3 : HepG2 cell lysate

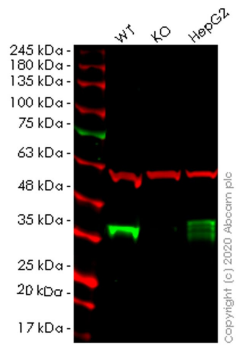
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 36 kDa

Lanes 1-3: Merged signal (red and green). Green - **ab109177** observed at 36 kDa. Red - loading control **ab7291** observed at 50 kDa.

ab109177 Anti-NEK6 antibody [EPR5282] was shown to specifically react with NEK6 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265910 (knockout cell lysate **ab258072**) was used. Wild-type and NEK6 knockout samples were subjected to SDS-PAGE. **ab109177** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) 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 NEK6 knockout HeLa cell line (ab265910)

All lanes : Anti-NEK6 antibody [EPR5283] (**ab133494**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : NEK6 knockout HeLa cell lysate
Lane 3 : HepG2 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

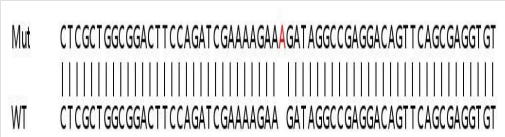
Predicted band size: 36 kDa
Observed band size: 36 kDa

Lanes 1-3: Merged signal (red and green). Green - **ab133494** observed at 36 kDa. Red - loading control **ab7291** observed at 50 kDa.

ab133494 Anti-NEK6 antibody [EPR5283] was shown to specifically react with NEK6 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265910 (knockout cell lysate **ab258072**) was used. Wild-type and NEK6 knockout samples were subjected to SDS-PAGE. **ab133494** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) 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.

Sanger Sequencing - Human NEK6 knockout HeLa cell line (ab265910)

Allele-1: 304 bp deletion in exon 3.



Sanger Sequencing - Human NEK6 knockout HeLa cell line (ab265910)

Allele-2: 1 bp insertion in exon 3.

Sanger Sequencing - Human NEK6 knockout HeLa cell line (ab265910)

Allele-3: Insertion of the selection cassette in exon 3.

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