abcam

Product datasheet

Human HTT (Huntingtin) knockout HeLa cell line ab265976

4 图像

概述

产品名称	人HTT (Huntingtin) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp deletion in exon 21
Passage number	<20
Knockout validation	Sanger Sequencing
经测试应用	**适用于:** WB
Biosafety level	2
常规说明	Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images. **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.
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.

性能

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
Antibiotic resistance	Puromycin 1.00µg/ml
Mycoplasma free	Yes
存放说明	Shipped on Dry Ice. Store in liquid nitrogen.
存储溶液	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

靶标

功能	May play a role in microtubule-mediated transport or vesicle function.
组织特异性	Expressed in the brain cortex (at protein level). Widely expressed with the highest level of expression in the brain (nerve fibers, varicosities, and nerve endings). In the brain, the regions where it can be mainly found are the cerebellar cortex, the neocortex, the striatum, and the hippocampal formation.
疾病相关	Defects in HTT are the cause of Huntington disease (HD) [MIM:143100]. HD is an autosomal dominant neurodegenerative disorder characterized by involuntary movements (chorea), general motor impairment, psychiatric disorders and dementia. Onset of the disease occurs usually in the third or fourth decade of life and symptoms progressively worsen leading to death in 10 to 20 years. Onset and clinical course depend on the degree of poly-Gln repeat expansion, longer expansions resulting in earlier onset and more severe clinical manifestations. HD affects 1 in 10,000 individuals of European origin. Neuropathology of Huntington disease displays a distinctive pattern with loss of neurons, especially in the caudate and putamen (striatum).
序列相似性	Belongs to the huntingtin family. Contains 10 HEAT repeats.
结构域	The N-terminal Gln-rich and Pro-rich domain has great conformational flexibility and is likely to exist in a fluctuating equilibrium of alpha-helical, random coil, and extended conformations.
翻译后修饰	Cleaved by apopain downstream of the polyglutamine stretch. The resulting N-terminal fragment is cytotoxic and provokes apoptosis.

	Forms with expanded polyglutamine expansion are specifically ubiquitinated by SYVN1, which promotes their proteasomal degradation.
细胞定位	Cytoplasm. Nucleus. The mutant Huntingtin protein colocalizes with AKAP8L in the nuclear matrix of Huntington's disease neurons.

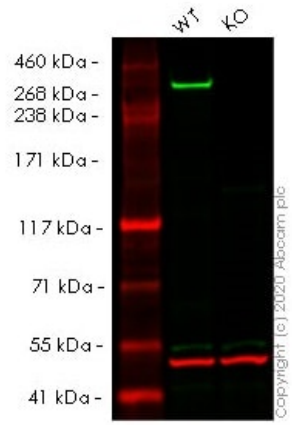
应用

The Abpromise guarantee **Abpromise™承诺保证使用ab265976于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		Use at an assay dependent concentration. Predicted molecular weight: 348 kDa. Western blot data indicates that the CRISPR gene edit may have resulted in a truncation of the protein of interest. Please see data images.

图片



Western blot - Human HTT (Huntingtin) knockout HeLa cell line (ab265976)

All lanes : Anti-Huntingtin antibody [EPR5526] (**ab109115**) at 1/10000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : HTT knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

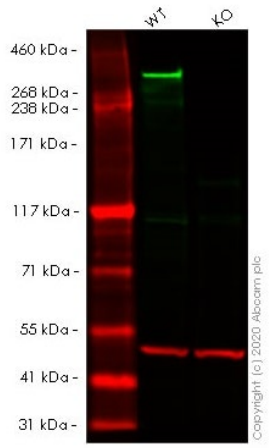
Performed under reducing conditions.

Predicted band size: 348 kDa
Observed band size: 348 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab109115** observed at 348 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

ab109115 was shown to react with Huntingtin in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265976 (knockout cell lysate **ab256946**) was used. Wild-type HeLa and HTT knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab109115**

and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) overnight at 4°C at a 1 in 10000 dilution and a 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 HTT (Huntingtin) knockout HeLa cell line (ab265976)

All lanes : Anti-Huntingtin antibody [EP867Y] (**ab45169**) at 1/10000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : HTT knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

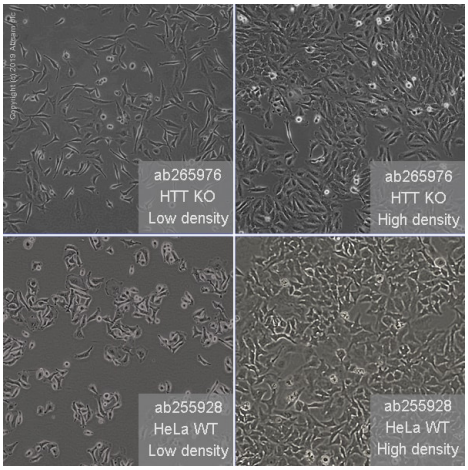
Predicted band size: 348 kDa
Observed band size: 348 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab45169** observed at 348 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

ab45169 was shown to react with Huntingtin in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265976 (knockout cell lysate **ab256946**) was used. Wild-type HeLa and HTT knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab45169** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) overnight at 4°C at a 1 in 10000 dilution and a 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 HTT knockout HeLa cell line (ab265976)

Homozygous: 1 bp deletion in exon 21.



Cell Culture - Human HTT (Huntingtin) knockout HeLa cell line (ab265976)

Representative images of HTT knockout HeLa cells, low and high confluency examples (top left and right respectively) and wild-type HeLa cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using a EVOS XL Core microscope.

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