abcam

Product datasheet

Human COMT knockout HEK-293T cell line ab266537

4 Images

Overview

Product name	Human COMT knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 3
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**Recommended control:** Human wild-type HEK293T cell line (**ab255449**). 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.

Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Kidney
Cell type	epithelial
STR Analysis	Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12
Antibiotic resistance	Puromycin 1.00µg/ml
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	Catalyzes the O-methylation, and thereby the inactivation, of catecholamine neurotransmitters and catechol hormones. Also shortens the biological half-lives of certain neuroactive drugs, like L-DOPA, alpha-methyl DOPA and isoproterenol.
Tissue specificity	Brain, liver, placenta, lymphocytes and erythrocytes.
Sequence similarities	Belongs to the mammalian catechol-O-methyltransferase family.
Post-translational modifications	The N-terminus is blocked.
Cellular localization	Cytoplasm and Cell membrane.

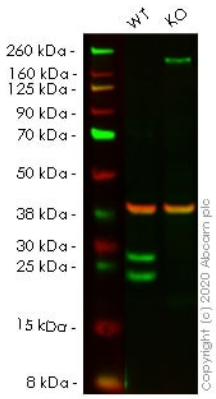
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab266537 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: 30 kDa.

Images



Western blot - Human COMT knockout HEK293T cell line (ab266537)

All lanes : Anti-COMT antibody [EPR6490] (**ab126618**) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : COMT knockout HEK-293T cell lysate

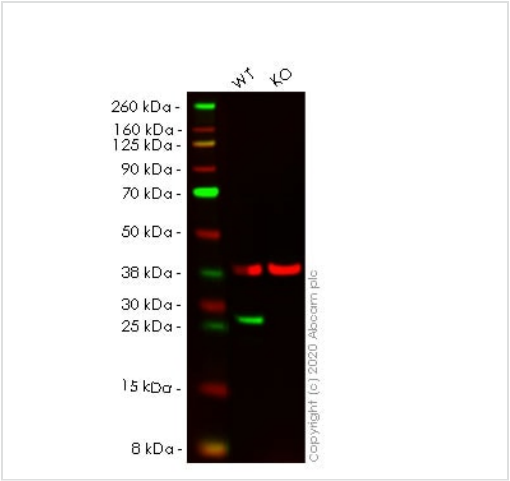
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 30 kDa
Observed band size: 24-28 kDa

Lanes 1-2: Merged signal (red and green). Green - **ab126618** observed at 24-28 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab126618 Anti-COMT antibody [EPR6490] was shown to specifically react with COMT in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab266537 (knockout cell lysate **ab257396**) was used. Wild-type and COMT knockout samples were subjected to SDS-PAGE. **ab126618** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) 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 COMT knockout HEK293T cell line (ab266537)

All lanes : Anti-COMT antibody [EPR6491(B)] (**ab124813**) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : COMT knockout HEK-293T cell lysate

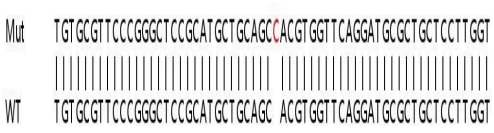
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 30 kDa
Observed band size: 28 kDa

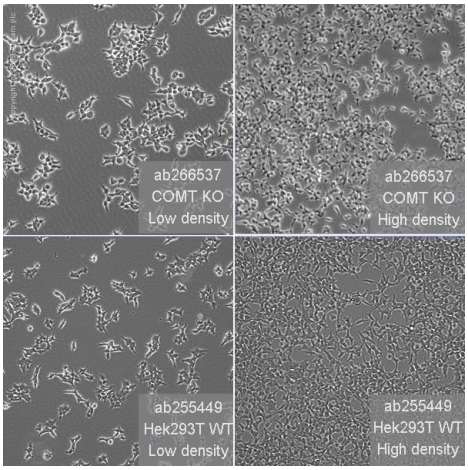
Lanes 1-2: Merged signal (red and green). Green - **ab124813** observed at 28 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab124813 Anti-COMT antibody [EPR6491(B)] was shown to specifically react with COMT in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab266537 (knockout cell lysate **ab257396**) was used. Wild-type and COMT knockout samples were subjected to SDS-PAGE. **ab124813** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) 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 COMT knockout HEK293T cell line (ab266537)

Homozygous: 1 bp insertion in exon 3



Cell Culture - Human COMT knockout HEK293T cell line (ab266537)

Representative images of COMT knockout HEK293T cells, low and high confluency examples (top left and right respectively) and wild-type HEK293T 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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