

Product datasheet

Human COMT knockout HEK-293T cell lysate ab257396

3 Images

Overview

Product name	Human COMT knockout HEK-293T cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
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)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT.

**Usage of SDS sample buffer is not recommended with these lyophilized lysates.*

Notes

Lysate preparation: Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found [here](#). Please refer to our lysis protocol for further details on how our lysates are prepared.

User storage instructions: Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage.

Access thousands of knockout cell lysates, generated from commonly used cancer cell lines.

[See here for more information on knockout cell lysates.](#)

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It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

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Tested applications

Suitable for: WB

Properties

Storage instructions Store at -80°C. Please refer to protocols.

Components	1 kit
ab260219 - Human COMT knockout HEK293T cell lysate	1 x 100µg
ab255553 - Human wild-type HEK293T cell lysate	1 x 100µg

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

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.

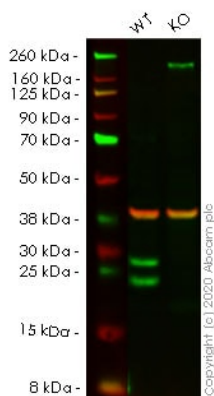
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257396 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



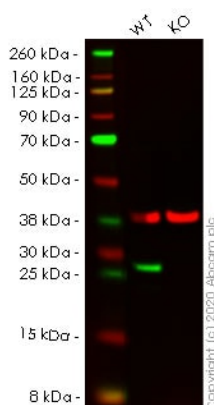
Western blot - Human COMT knockout HEK293T cell lysate (ab257396)

Lane 1: Wild-type HEK-293T cell lysate (20 µg)

Lane 2: COMT knockout HEK-293T cell lysate (20 µg)

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 lysate (ab257396)

Lane 1: Wild-type HEK-293T cell lysate (20 µg)

Lane 2: COMT knockout HEK-293T cell lysate (20 µg)

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.

Mut	TGTGGTTCCCGGGCTCCGCATGCTGCAGC	ACGTGGTTCAGGATGCCTGCTCCTTGGT
WT	TGTGGTTCCCGGGCTCCGCATGCTGCAGC	ACGTGGTTCAGGATGCCTGCTCCTTGGT

Sanger Sequencing - Human COMT knockout
HEK293T cell lysate (ab257396)

Homozygous: 1 bp insertion in exon 3

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