abcam

Product datasheet

Human PTTG1 (Securin) knockout HEK-293T cell lysate ab257289

3 Images

Overview

Product name	Human PTTG1 (Securin) 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: 8 bp deletion in exon 1.
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.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. 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. 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**.
Tested applications	**Suitable for:** WB

Properties

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

Components	1 kit
ab260172 - Human PTTG1 knockout HEK293T cell lysate	1 x 100µg
ab255553 - Human wild-type HEK293T cell lysate	1 x 100µg

Cell type epithelial

Gender Female

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 Regulatory protein, which plays a central role in chromosome stability, in the p53/TP53 pathway, and DNA repair. Probably acts by blocking the action of key proteins. During the mitosis, it blocks Separase/ESPL1 function, preventing the proteolysis of the cohesin complex and the subsequent segregation of the chromosomes. At the onset of anaphase, it is ubiquitinated, conducting to its destruction and to the liberation of ESPL1. Its function is however not limited to a blocking activity, since it is required to activate ESPL1. Negatively regulates the transcriptional activity and related apoptosis activity of TP53. The negative regulation of TP53 may explain the strong transforming capability of the protein when it is overexpressed. May also play a role in DNA repair via its interaction with Ku, possibly by connecting DNA damage-response pathways with sister chromatid separation.

Tissue specificity Expressed at low level in most tissues, except in adult testis, where it is highly expressed. Overexpressed in many patients suffering from pituitary adenomas, primary epithelial neoplasias, and esophageal cancer.

Sequence similarities Belongs to the securin family.

Developmental stage Low level during G1 and S phases. Peaks at M phase. During anaphase, it is degraded.

Domain The N-terminal destruction box (D-box) acts as a recognition signal for degradation via the ubiquitin-proteasome pathway.
The TEK-boxes are required for 'Lys-11'-linked ubiquitination and facilitate the transfer of the first ubiquitin and ubiquitin chain nucleation. TEK-boxes may direct a catalytically competent orientation of the UBE2C/UBCH10-ubiquitin thiolester with the acceptor lysine residue.

Post-translational modifications Phosphorylated at Ser-165 by CDK1 during mitosis.
Phosphorylated in vitro by ds-DNA kinase.
Ubiquitinated through 'Lys-11' linkage of ubiquitin moieties by the anaphase promoting complex (APC) at the onset of anaphase, conducting to its degradation. 'Lys-11'-linked ubiquitination is mediated by the E2 ligase UBE2C/UBCH10.

Cellular localization Cytoplasm. Nucleus.

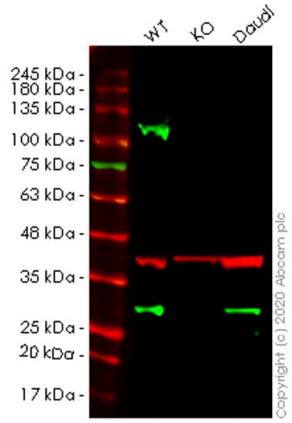
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257289 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: 22 kDa.

Images



Western blot - Human PTTG1 knockout HEK293T cell lysate (ab257289)

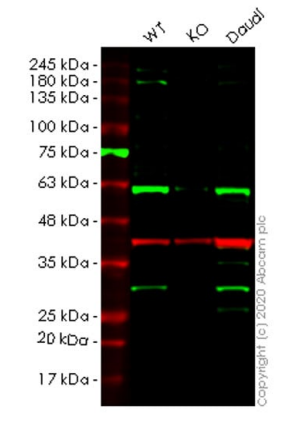
Lane 1: Wild-type HEK293T cell lysate (20 µg)

Lane 2: PTTG1 knockout HEK293T cell lysate (20 µg)

Lane 3: Daudi cell lysate (20 µg)

Lanes 1-3: Merged signal (red and green). Green - **ab79546** observed at 28 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab79546 Anti-Securin antibody [EPR3240] was shown to specifically react with Securin in wild-type HEK293T cells. Loss of signal was observed when knockout cell line **ab266231** (knockout cell lysate ab257289) was used. Wild-type and Securin knockout samples were subjected to SDS-PAGE. **ab79546** 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 10000 dilution for 1 hour at room temperature before imaging.



Western blot - Human PTTG1 knockout HEK293T cell lysate (ab257289)

Lane 1: Wild-type HEK293T cell lysate (20 µg)

Lane 2: PTTG1 knockout HEK293T cell lysate (20 µg)

Lane 3: Daudi cell lysate (20 µg)

Lanes 1-3: Merged signal (red and green). Green - **ab26273** observed at 28 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab26273 Anti-Securin antibody was shown to specifically react with Securin in wild-type HEK293T cells. Loss of signal was observed when knockout cell line **ab266231** (knockout cell lysate ab257289) was used. Wild-type and Securin knockout samples were subjected to SDS-PAGE. **ab26273** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 500 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 10000 dilution for 1 hour at room temperature before imaging.

Sanger Sequencing - Human PTTG1 knockout HEK293T cell lysate (ab257289)

Homozygous: 8 bp deletion in exon 1

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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