abcam

Product datasheet

Human ITPA knockout HEK-293T cell lysate ab258477

3 Images

Overview

Product name	Human ITPA 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 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, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and 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
ab261253 - Human ITPA 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 Hydrolyzes ITP and dITP to their respective monophosphate derivatives. Xanthosine 5'-triphosphate (XTP) is also a potential substrate. May be the major enzyme responsible for regulating ITP concentration in cells.

Tissue specificity Ubiquitous. Highly expressed in heart, liver, sex glands, thyroid and adrenal gland.

Involvement in disease Defects in ITPA are the cause of inosine triphosphate pyrophosphohydrolase deficiency (ITPA deficiency) [MIM:147520]. It is a common inherited trait characterized by the abnormal accumulation of inosine triphosphate (ITP) in erythrocytes and also leukocytes and fibroblasts. The pathological consequences of ITPA deficiency, if any, are unknown. However, it might have pharmacogenomic implications and be related to increased drug toxicity of purine analog drugs. Three different human populations have been reported with respect to their ITPase activity: high, mean (25% of high) and low activity. The variant Thr-32 is associated with complete loss of enzyme activity, may be by altering the local secondary structure of the protein. Heterozygotes for this polymorphism have 22.5% of the control activity: this is consistent with a dimeric structure of the enzyme.

Sequence similarities Belongs to the HAM1 NTPase family.

Cellular localization Cytoplasm.

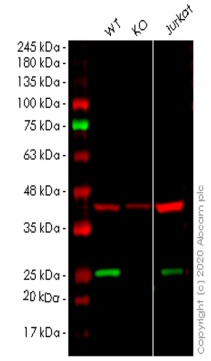
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab258477 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: 21 kDa.

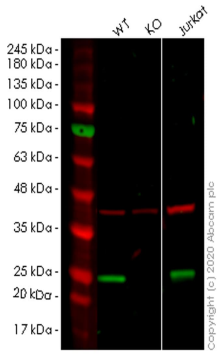
Images



Western blot - Human ITPA knockout HEK293T cell lysate (ab258477)

Lane 1: Wild-type HEK293T cell lysate (20 ug)
Lane 2: ITPA knockout HEK293T cell lysate (20 ug)
Lane 3: Jurkat cell lysate (20 ug)

ab150420 was shown to specifically react with ITPA in wild-type HEK293T cells. Loss of signal was observed when knockout cell line **ab266679** (knockout cell lysate ab258477) was used. Wild-type and ITPA knockout samples were subjected to SDS-PAGE. **ab150420** 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.



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ab134937 was shown to specifically react with ITPA in wild-type HEK293T cells. Loss of signal was observed when knockout cell line **ab266679** (knockout cell lysate ab258477) was used. Wild-type and ITPA knockout samples were subjected to SDS-PAGE. **ab134937** 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 ITPA knockout HEK293T cell lysate (ab258477)

Homozygous: 1 bp insertion in exon 1

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

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