abcam

Product datasheet

Human GLYR1 knockout HEK-293T cell lysate ab257968

3 Images

Overview

Product name	Human GLYR1 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: 10 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.** 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
ab261112 - Human GLYR1 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 May have oxidoreductase activity. Regulates p38 MAP kinase activity by mediating stress activation of p38alpha/MAPK14 and specifically regulating MAPK14 signaling. Indirectly promotes phosphorylation of MAPK14 and activation of ATF2. The phosphorylation of MAPK14 requires upstream activity of MAP2K4 and MAP2K6. Recruited on chromatin, recognizes and binds trimethylated 'Lys-36' of histone H3 (H3K36me3).

Sequence similarities Belongs to the 3-hydroxyisobutyrate dehydrogenase family. NP60 subfamily.
Contains 1 A.T hook DNA-binding domain.
Contains 1 PWWP domain.

Domain The A.T hook DNA-binding domain is required for the interaction with MAPK14.
The PWWP domain probably mediates the binding to H3K36me3.

Cellular localization Nucleus.

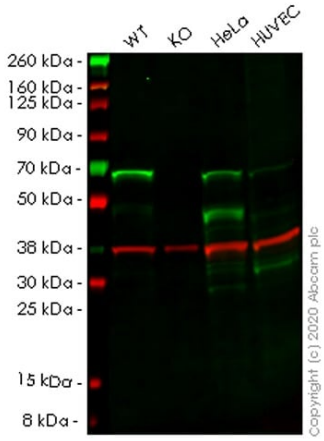
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab257968 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: 61 kDa.

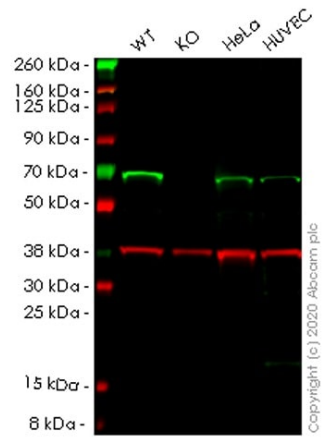
Images



Western blot - Human GLYR1 knockout HEK293T cell lysate (ab257968)

Lane 1: Wild-type HEK293T cell lysate (20 ug)
Lane 2: GLYR1 knockout HEK293T cell lysate (20 ug)
Lane 3: HeLa cell lysate (20 ug)
Lane 4: HUVEC cell lysate (20 ug)

ab167155 was shown to specifically react with GLYR1 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line **ab266610** (knockout cell lysate ab257968) was used. Wild-type and GLYR1 knockout samples were subjected to SDS-PAGE. **ab167155** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4^{o}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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ab154838 was shown to specifically react with GLYR1 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line **ab266610** (knockout cell lysate ab257968) was used. Wild-type and GLYR1 knockout samples were subjected to SDS-PAGE. **ab154838** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4^{o}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 GLYR1 knockout

HEK293T cell lysate (ab257968)

Homozygous: 10 bp insertion in exon 3

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

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