abcam

Product datasheet

Human CBLB knockout HeLa cell lysate ab258342

2 Images

Overview

Product name	Human CBLB knockout HeLa cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon6.
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
ab262384 - Human CBLB knockout HeLa cell lysate	1 x 100µg
ab255929 - Human wild-type HeLa cell lysate	1 x 100µg

Cell type epithelial

Disease Adenocarcinoma

Gender Female

STR Analysis Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8,12 CSF1PO: 9, 10

Target

Relevance Function: E3 ubiquitin-protein ligase which accepts ubiquitin from specific E2 ubiquitin-conjugating enzymes, and transfers it to substrates, generally promoting their degradation by the proteasome. Negatively regulates TCR (T-cell receptor), BCR (B-cell receptor) and FCER1 (high affinity immunoglobulin epsilon receptor) signal transduction pathways. In naive T-cells, inhibits VAV1 activation upon TCR engagement and imposes a requirement for CD28 costimulation for proliferation and IL-2 production. Also acts by promoting PIK3R1/p85 ubiquitination, which impairs its recruitment to the TCR and subsequent activation. In activated T-cells, inhibits PLCG1 activation and calcium mobilization upon restimulation and promotes anergy. In B-cells, acts by ubiquitinating SYK and promoting its proteasomal degradation. Slightly promotes SRC ubiquitination. May be involved in EGFR ubiquitination and internalization. May be functionally coupled with the E2 ubiquitin-protein ligase UB2D3. Tissue specificity: Expressed in placenta, heart, lung, kidney, spleen, ovary and testis, as well as fetal brain and liver and hematopoietic cell lines, but not in adult brain, liver, pancreas, salivary gland, or skeletal muscle. Present in lymphocytes (at protein level). Pathway: Protein modification; protein ubiquitination. Similarity: Contains 1 Cbl-PTB (Cbl-type phosphotyrosine-binding) domain. Contains 1 RING-type zinc finger. Contains 1 UBA domain. Domain: The N-terminus is composed of the phosphotyrosine binding (PTB) domain, a short linker region and the RING-type zinc finger. The PTB domain, which is also called TKB (tyrosine kinase binding) domain, is composed of three different subdomains: a four-helix bundle (4H), a calcium-binding EF hand and a divergent SH2 domain. The RING-type zinc finger domain mediates binding to an E2 ubiquitin-conjugating enzyme. The UBA domain interacts with poly-ubiquitinated proteins. PTM: Phosphorylated on tyrosine and serine residues upon TCR or BCR activation, and upon various types of cell stimulation. Auto-ubiquitinated upon EGF-mediated cell activation or upon T-cell costimulation by CD28; which promotes proteasomal degradation.

Cellular localization Cytoplasmic and Nuclear

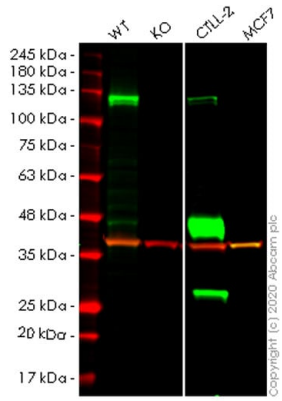
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab258342 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: 109 kDa.

Images



Western blot - Human CBLB knockout HeLa cell lysate (ab258342)

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

Lane 2: CBLB knockout HeLa cell lysate (20 µg)

Lane 3: CTLL-2 cell lysate (20 µg)

Lane 4: MCF7 cell lysate (20 µg)

Lanes 1-4: Merged signal (red and green). Green - **ab54362** observed at 130 kDa. Red - loading control, **ab181602** observed at 37 kDa.

ab54362 Anti-CBLB antibody [246C5a] was shown to specifically react with CBLB in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab264711** (knockout cell lysate ab258342) was used. Wild-type and CBLB knockout samples were subjected to SDS-PAGE. **ab54362** and Anti-GAPDH antibody [EPR16891] - Loading Control (**ab181602**) were incubated overnight at 4°C at 1 in 500 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.

Sanger Sequencing - Human CBLB knockout HeLa cell lysate (ab258342)

Homozygous: 1 bp deletion in exon6

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