

Human OTUB1 knockout HEK-293T cell lysate ab257569

画像数 4

製品の概要

製品名	Human OTUB1 knockout HEK-293T cell lysate
製品の概要	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 19 bp deletion in exon 1 and Insertion of the selection cassette 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. <i>*Usage of SDS sample buffer is not recommended with these lyophilized lysates.</i>

特記事項

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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アプリケーション

適用あり: WB

製品の特性

保存方法 Store at -80°C. Please refer to protocols.

内容	1 kit
ab260285 - Human OTUB1 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

ターゲット情報

機能 Hydrolase that can remove conjugated ubiquitin from proteins and plays an important regulatory role at the level of protein turnover by preventing degradation. Regulator of T-cell anergy, a phenomenon that occurs when T-cells are rendered unresponsive to antigen rechallenge and no longer respond to their cognate antigen. Acts via its interaction with RNF128/GRAIL, a crucial inductor of CD4 T-cell anergy. Isoform 1 destabilizes RNF128, leading to prevent anergy. In contrast, isoform 2 stabilizes RNF128 and promotes anergy. Surprisingly, it regulates RNF128-mediated ubiquitination, but does not deubiquitinate polyubiquitinated RNF128. Deubiquitinates estrogen receptor alpha (ESR1). Mediates deubiquitination of 'Lys-48'-linked polyubiquitin chains, but not 'Lys-63'-linked polyubiquitin chains. Not able to cleave di-ubiquitin. Also capable of removing NEDD8 from NEDD8 conjugates, but with a much lower preference compared to 'Lys-48'-linked ubiquitin.

組織特異性 Isoform 1 is ubiquitous. Isoform 2 is expressed only in lymphoid tissues such as tonsils, lymph nodes and spleen, as well as peripheral blood mononuclear cells.

配列類似性 Belongs to the peptidase C65 family.
Contains 1 OTU domain.

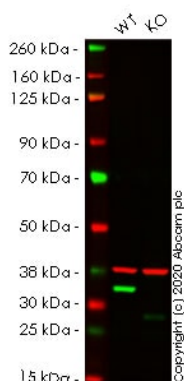
ドメイン In addition to ubiquitin-binding at the Cys-91 active site, a proximal ubiquitin-binding site is also present at Cys-23. Occupancy of the active site is needed to enable tight binding to the second site. Distinct binding sites for the ubiquitins may allow to discriminate among different isopeptide linkages (i.e. 'Lys-48'-, 'Lys-63'-linked polyubiquitin) in polyubiquitin substrates and achieve linkage-specific deubiquitination.

細胞内局在 Cytoplasm.

アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab257569の使用に適用されます
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 31 kDa.



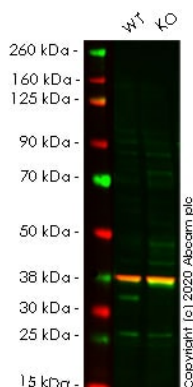
Western blot - Human OTUB1 knockout HEK293T cell lysate (ab257569)

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

Lane 2: OTUB1 knockout HEK-293T cell lysate (20 µg)

Lanes 1-2: Merged signal (red and green). Green - [ab175200](#) observed at 31 kDa. Red - loading control [ab8245](#) observed at 37 kDa.

[ab175200](#) Anti-OTUB1 antibody [EPR13028(B)] was shown to specifically react with OTUB1 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line [ab266551](#) (knockout cell lysate ab257569) was used. Wild-type and OTUB1 knockout samples were subjected to SDS-PAGE. [ab175200](#) 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 OTUB1 knockout HEK293T cell lysate (ab257569)

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

Lane 2: OTUB1 knockout HEK-293T cell lysate (20 µg)

Lanes 1-2: Merged signal (red and green). Green - [ab101471](#) observed at 130 kDa. Red - loading control [ab8245](#) observed at 37 kDa.

[ab101471](#) Anti-OTUB1 antibody was shown to specifically react with OTUB1 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line [ab266551](#) (knockout cell lysate ab257569) was used. Wild-type and OTUB1 knockout samples were subjected to SDS-PAGE. [ab101471](#) and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) were incubated overnight at 4°C at 1 in 10000 ug/ml 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	GGT CGGT AGT GCGGCG-----GAGGAACCTCAGCAGCAGAAGCAGG
WT	GGT CGGT AGT GCGGCGCTGTTTAAAGATGGCGGCGGAGGAACCTCAGCAGCAGAAGCAGG

Allele-1: 19 bp deletion in exon 1

Sanger Sequencing - Human OTUB1 knockout

HEK293T cell lysate (ab257569)

Mut	AAGCGGT CGGT AGT GCGGCG****Insertion****CTGTTTAAAGATGGCGGCGG
WT	AAGCGGT CGGT AGT GCGGCGCTGTTTAAAGATGGCGGCGG

Allele-2: Insertion of the selection cassette in exon 1

Sanger Sequencing - Human OTUB1 knockout

HEK293T cell lysate (ab257569)

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