abcam

Product datasheet

Human LCK knockout Jurkat cell line ab273855

画像数 4

製品の概要

製品名	Human LCK knockout Jurkat cell line
Parental Cell Line	Jurkat
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 1 bp insertion, 2 bp insertion; Frameshift: 99%
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS), Western Blot (WB)
アプリケーション	**適用あり:** Next Generation Sequencing, WB
Biosafety level	1
特記事項	**Recommended control:** Human wild-type Jurkat cell line (**ab271143**). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** RPMI + 10% FBS **Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability. 1. Thaw the vial in 37°C water for bath approximately 1-2 minutes. 2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution. 3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $1x10^5$ cells/mL. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $1x10^5$ cells/mL is recommended. Do not allow the cell density to exceed $3x10^6$. 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**.

We will provide viable cells that proliferate on revival.

製品の特性

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Suspension
Tissue	Blood
Cell type	T cell lymphoblast-like
Disease	Non-Hodgkin Lymphoma
Gender	Male
Mycoplasma free	Yes
保存方法	Shipped on Dry Ice. Store in liquid nitrogen.
バッファー	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

ターゲット情報

機能	Tyrosine kinase that plays an essential role for the selection and maturation of developing T-cell in the thymus and in mature T-cell function. Is constitutively associated with the cytoplasmic portions of the CD4 and CD8 surface receptors and plays a key role in T-cell antigen receptor(TCR)-linked signal transduction pathways. Association of the TCR with a peptide antigen-bound MHC complex facilitates the interaction of CD4 and CD8 with MHC class II and class I molecules, respectively, and thereby recruits the associated LCK to the vicinity of the TCR/CD3 complex. LCK then phosphorylates tyrosines residues within the immunoreceptor tyrosines-based activation motifs (ITAMs) in the cytoplasmic tails of the TCRgamma chains and CD3 subunits, initiating the TCR/CD3 signaling pathway. In addition, contributes to signaling by other receptor molecules. Associates directly with the cytoplasmic tail of CD2, and upon engagement of the CD2 molecule, LCK undergoes hyperphosphorylation and activation. Also plays a role in the IL2 receptor-linked signaling pathway that controls T-cell proliferative response. Binding of IL2 to its receptor results in increased activity of LCK. Is expressed at all stages of thymocyte development and is required for the regulation of maturation events that are governed by both pre-TCR and mature alpha beta TCR. Phosphorylates RUNX3.
組織特異性	Expressed specifically in lymphoid cells.
関連疾患	Note=A chromosomal aberration involving LCK is found in leukemias. Translocation t(1;7) (p34;q34) with TCRB.
配列類似性	Belongs to the protein kinase superfamily. Tyr protein kinase family. SRC subfamily. Contains 1 protein kinase domain. Contains 1 SH2 domain. Contains 1 SH3 domain.
ドメイン	The SH2 domain mediates interaction with SQSTM1. Interaction is regulated by Ser-59 phosphorylation.
翻訳後修飾	Phosphorylated on Tyr-394, which increases enzymatic activity (By similarity). Phosphorylated on Tyr-505, which decreases activity.

細胞内局在	Cytoplasm. Cell membrane. Present in lipid rafts in an unactive form.
製品の状態	This protein is known to be similar in amino acid sequence to HCK (P08631), FYN (P06241), YES1 (P07947), SRC (P12931), and LYN (P07948). Therefore, cross-reactivity with these homologous proteins may be observed. We would be happy to provide immunogen alignment information upon request.

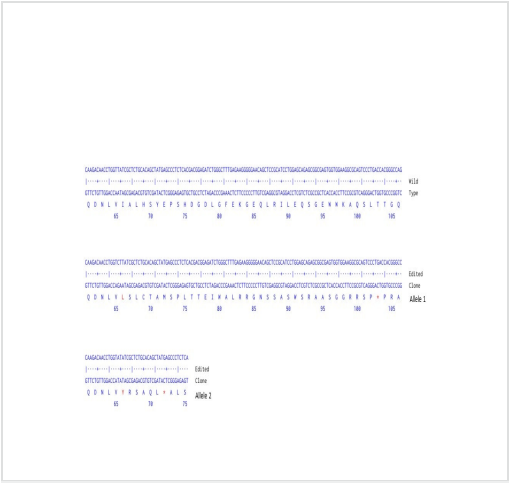
アプリケーション

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ab273855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。

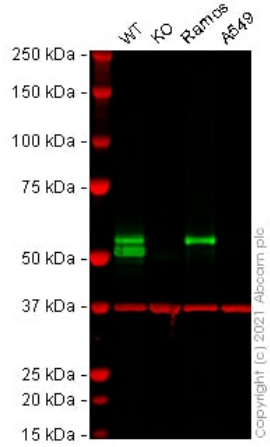
アプリケーション	Abreviews	特記事項
Next Generation Sequencing		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 58 kDa.

画像



Next Generation Sequencing - Human LCK knockout Jurkat cell line (ab273855)

2 bp insertion (allele 1) and 1 bp insertion (allele 2) after Val65 of the WT protein



Western blot - Human LCK knockout Jurkat cell line (ab273855)

All lanes : Anti-Lck antibody [EPR20798-107] (**ab227975**) at 1/1000 dilution

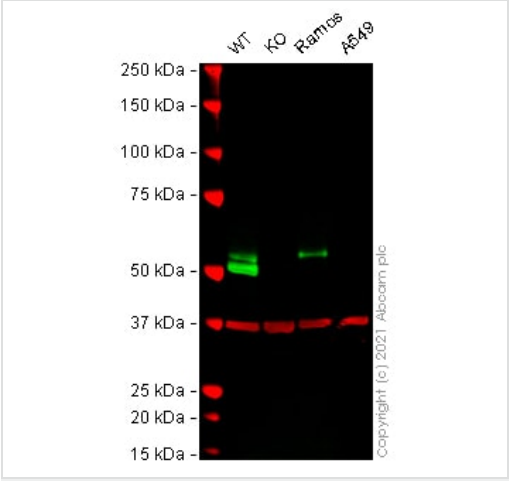
Lane 1 : Wild-type Jurkat cell lysate
Lane 2 : Lck knockout Jurkat cell lysate
Lane 3 : Ramos cell lysate
Lane 4 : A549 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 58 kDa
Observed band size: 60 kDa

False colour image of Western blot: Anti-Lck antibody [EPR20798-107] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab227975** was shown to bind specifically to Lck. A band was observed at 60 kDa in wild-type Jurkat cell lysates with no signal observed at this size in Lck knockout cell line ab273855 (knockout cell lysate **ab273809**). To generate this image, wild-type and Lck knockout Jurkat cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Human LCK knockout Jurkat cell line (ab273855)

All lanes : Anti-Lck antibody [Y123] (**ab32149**) at 1/1000 dilution

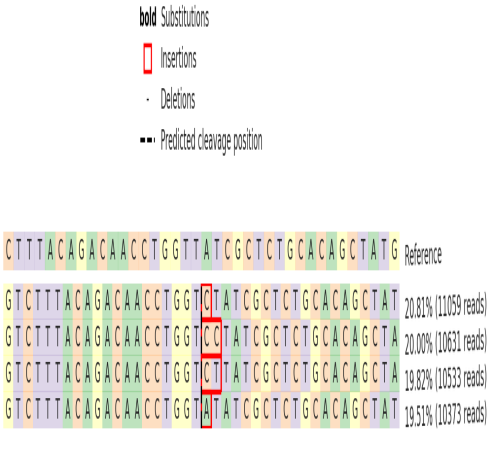
Lane 1 : Wild-type Jurkat cell lysate
Lane 2 : Lck knockout Jurkat cell lysate
Lane 3 : Ramos cell lysate
Lane 4 : A549 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 58 kDa
Observed band size: 60 kDa

False colour image of Western blot: Anti-Lck antibody [Y123] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab32149** was shown to bind specifically to Lck. A band was observed at 60 kDa in wild-type Jurkat cell lysates with no signal observed at this size in Lck knockout cell line ab273855 (knockout cell lysate **ab273809**). To generate this image, wild-type and Lck knockout Jurkat cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Next Generation Sequencing - Human LCK knockout Jurkat cell line (ab273855)

Knockout achieved by CRISPR/Cas9; X = 1 bp insertion, 2 bp insertion; Frameshift: 99%

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