abcam

Product datasheet

Human JAK2 knockout A549 cell line ab267113

画像数 3

製品の概要

製品名	Human JAK2 knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 3 and 5 bp deletion in exon 3
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
アプリケーション	**適用あり:** WB
Biosafety level	2
特記事項	**Recommended control:** Human wild-type A549 cell line (**ab255450**). 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:** F-12K + 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 bath for 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 $2x10^3$-$1x10^4$ cells/cm^2. 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 $6x10^4$ cells/cm^2 is recommended. A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.

Cells should be passaged when they have achieved 80-90% confluence.
Do not exceed $7x10^4$ cells/cm^2.
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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	Adherent
Tissue	Lung
Cell type	epithelial
Disease	Carcinoma
Gender	Male
STR Analysis	Amelogenin X,Y D5S818: 11 D13S317: 11 D7S820: 8, 11 D16S539: 11, 12 vWA: 14 TH01: 8,9.3 TPOX: 8,11 CSF1PO: 10, 12
Antibiotic resistance	Puromycin 1.00µg/ml
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	Non-receptor tyrosine kinase involved in various processes such as cell cycle progression, apoptosis, mitotic recombination, genetic instability and histone modifications. In the cytoplasm, plays a pivotal role in signal transduction via its association with cytokine receptors, which constitutes an initiating step in signaling for many members of the cytokine receptor superfamily including the receptors for growth hormone (GHR), prolactin (PRLR), leptin (LEPR), erythropoietin (EPOR), granulocyte-macrophage colony-stimulating factor (CSF2), thrombopoietin (THPO) and multiple interleukins. Following stimulation with erythropoietin (EPO) during erythropoiesis, it is autophosphorylated and activated, leading to its association with erythropoietin receptor (EPOR) and tyrosine phosphorylation of residues in the EPOR cytoplasmic domain. Also involved in promoting the localization of EPOR to the plasma membrane. Also acts downstream of some G-protein coupled receptors. Plays a role in the control of body weight (By similarity). Mediates angiotensin-2-induced ARHGEF1 phosphorylation. In the nucleus, plays a key role in chromatin by specifically mediating phosphorylation of 'Tyr-41' of histone H3 (H3Y41ph), a specific tag that promotes exclusion of CBX5 (HP1 alpha) from chromatin.
組織特異性	Expressed in blood, bone marrow and lymph node.
関連疾患	Note=Chromosomal aberrations involving JAK2 are found in both chronic and acute forms of eosinophilic, lymphoblastic and myeloid leukemia. Translocation t(8;9)(p22;p24) with PCM1 links the protein kinase domain of JAK2 to the major portion of PCM1. Translocation t(9;12)(p24;p13) with ETV6. Defects in JAK2 are a cause of susceptibility to Budd-Chiari syndrome (BCS) [MIM:600880]. It is a syndrome caused by obstruction of hepatic venous outflow involving either the hepatic veins or

	the terminal segment of the inferior vena cava. Obstructions are generally caused by thrombosis and lead to hepatic congestion and ischemic necrosis. Clinical manifestations observed in the majority of patients include hepatomegaly, right upper quadrant pain and abdominal ascites. Budd-Chiari syndrome is associated with a combination of disease states including primary myeloproliferative syndromes and thrombophilia due to factor V Leiden, protein C deficiency and antithrombin III deficiency. Budd-Chiari syndrome is a rare but typical complication in patients with polycythemia vera. Defects in JAK2 are a cause of polycythemia vera (PV) [MIM:263300]. A myeloproliferative disorder characterized by abnormal proliferation of all hematopoietic bone marrow elements, erythroid hyperplasia, an absolute increase in total blood volume, but also by myeloid leukocytosis, thrombocytosis and splenomegaly. Defects in JAK2 gene may be a cause of essential thrombocythemia (ET) [MIM:187950]. ET is characterized by elevated platelet levels due to sustained proliferation of megakaryocytes, and frequently lead to thrombotic and haemorrhagic complications. Defects in JAK2 are a cause of myelofibrosis (MYELOF) [MIM:254450]. Myelofibrosis is a disorder characterized by replacement of the bone marrow by fibrous tissue, occurring in association with a myeloproliferative disorder. Clinical manifestations may include anemia, pallor, splenomegaly, hypermetabolic state, petechiae, ecchymosis, bleeding, lymphadenopathy, hepatomegaly, portal hypertension. Defects in JAK2 are a cause of acute myelogenous leukemia (AML) [MIM:601626]. AML is a malignant disease in which hematopoietic precursors are arrested in an early stage of development.
配列類似性	Belongs to the protein kinase superfamily. Tyr protein kinase family. JAK subfamily. Contains 1 FERM domain. Contains 1 protein kinase domain. Contains 1 SH2 domain.
ドメイン	Possesses 2 protein kinase domains. The second one probably contains the catalytic domain, while the presence of slight differences suggest a different role for protein kinase 1.
翻訳後修飾	Autophosphorylated, leading to regulate its activity. Leptin promotes phosphorylation on tyrosine residues, including phosphorylation on Tyr-813. Autophosphorylation on Tyr-119 in response to EPO down-regulates its kinase activity. Autophosphorylation on Tyr-868, Tyr-966 and Tyr-972 in response to growth hormone (GH) are required for maximal kinase activity.
細胞内局在	Endomembrane system. Nucleus.

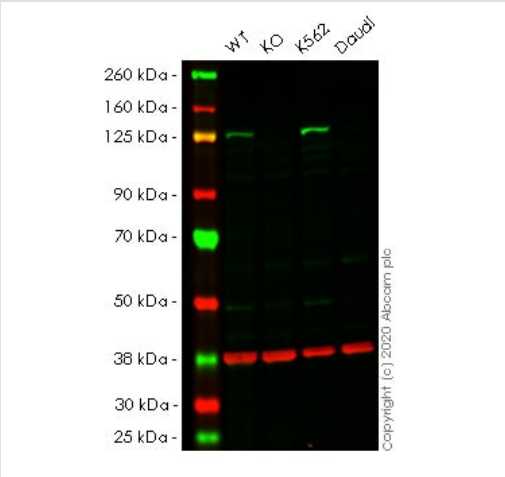
アプリケーション

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ab267113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: 131 kDa.

画像



Western blot - Human JAK2 knockout A549 cell line (ab267113)

All lanes : Anti-JAK2 antibody [EPR108(2)] (**ab108596**) at 1/1000 dilution

Lane 1 : Wild-type A549 cell lysate
Lane 2 : JAK2 knockout A549 cell lysate
Lane 3 : K562 cell lysate
Lane 4 : Daudi cell lysate

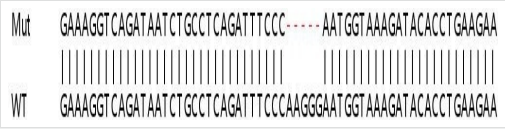
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 131 kDa
Observed band size: 131 kDa

Lanes 1- 4: Merged signal (red and green). Green - **ab108596** observed at 131 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab108596 was shown to react with JAK2 in wild-type A549 cells in western blot. Loss of signal was observed when knockout cell line ab267113 (knockout cell lysate **ab256963**) was used. Wild-type A549 and JAK2 knockout A549 cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab108596** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 dilution and a 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 JAK2 knockout A549 cell line (ab267113)

Allele-1: 5 bp deletion in exon3

Sanger Sequencing - Human JAK2 knockout A549 cell line (ab267113)

Allele-2: 1 bp insertion in exon 3.

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