

Human PTPN11 (SHP2) knockout HEK-293T cell line ab266450

5 图像

概述

产品名称	人PTPN11 (SHP2) knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 1 and 2 bp deletion in exon 1
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
经测试应用	适用于: WB
Biosafety level	2
常规说明	<p>Recommended control: Human wild-type HEK293T cell line (ab255449). 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.</p> <p>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: DMEM (High Glucose) + 10% FBS</p> <p>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.</p> <ol style="list-style-type: none"> 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 2×10^4 cells/cm². 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% CO₂. Cultures should be monitored daily. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of 2×10^4 cells/cm² is recommended.</p> <p>A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.</p>

Cells should be passaged when they have achieved 80-90% confluence.

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We will provide viable cells that proliferate on revival.

性能

Number of cells	1 x 10 ⁶ cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Kidney
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
Antibiotic resistance	Puromycin 1.00µg/ml
Mycoplasma free	Yes
存放说明	Shipped on Dry Ice. Store in liquid nitrogen.
存储溶液	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

靶标

功能	Acts downstream of various receptor and cytoplasmic protein tyrosine kinases to participate in the signal transduction from the cell surface to the nucleus.
组织特异性	Widely expressed, with highest levels in heart, brain, and skeletal muscle.
疾病相关	<p>Defects in PTPN11 are the cause of LEOPARD syndrome type 1 (LEOPARD1) [MIM:151100]. It is an autosomal dominant disorder allelic with Noonan syndrome. The acronym LEOPARD stands for lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonic stenosis, abnormalities of genitalia, retardation of growth, and deafness.</p> <p>Defects in PTPN11 are the cause of Noonan syndrome type 1 (NS1) [MIM:163950]. Noonan syndrome (NS) is a disorder characterized by dysmorphic facial features, short stature, hypertelorism, cardiac anomalies, deafness, motor delay, and a bleeding diathesis. Some patients with Noonan syndrome type 1 develop multiple giant cell lesions of the jaw or other bony or soft tissues, which are classified as pigmented villomoduolar synovitis (PVNS) when occurring in the jaw or joints. Note=Mutations in PTPN11 account for more than 50% of the cases. Rarely, NS is associated with juvenile myelomonocytic leukemia (JMML). NS1 inheritance is autosomal dominant.</p> <p>Defects in PTPN11 are a cause of juvenile myelomonocytic leukemia (JMML) [MIM:607785]. JMML is a pediatric myelodysplastic syndrome that constitutes approximately 30% of childhood cases of myelodysplastic syndrome (MDS) and 2% of leukemia. It is characterized by leukocytosis with tissue infiltration and in vitro hypersensitivity of myeloid progenitors to granulocyte-macrophage colony stimulating factor.</p> <p>Defects in PTPN11 are a cause of metachondromatosis (MC) [MIM:156250]. It is a skeletal disorder with radiologic fetarures of both multiple exostoses and Ollier disease, characterized by the presence of multiple enchondromas and osteochondroma-like lesions.</p>
序列相似性	Belongs to the protein-tyrosine phosphatase family. Non-receptor class 2 subfamily.

	Contains 2 SH2 domains. Contains 1 tyrosine-protein phosphatase domain.
结构域	The SH2 domains repress phosphatase activity. Binding of these domains to phosphotyrosine-containing proteins relieves this auto-inhibition, possibly by inducing a conformational change in the enzyme.
翻译后修饰	Phosphorylated on Tyr-546 and Tyr-584 upon receptor protein tyrosine kinase activation; which creates a binding site for GRB2 and other SH2-containing proteins.
细胞定位	Cytoplasm.

应用

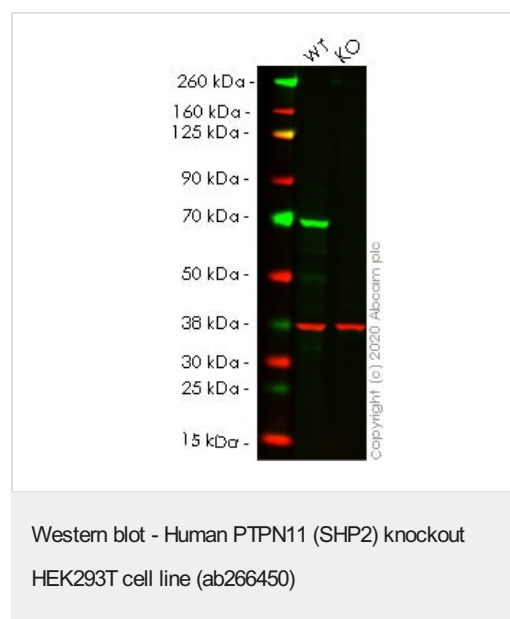
The Abpromise guarantee

Abpromise™ 承诺保证使用 ab266450 于以下的经测试应用

“应用说明”部分 下显示的仅为推荐的起始稀释度; 实际最佳的稀释度/浓度应由使用者检定。

应用	Ab 评论	说明
WB		Use at an assay dependent concentration. Predicted molecular weight: 68 kDa.

图片



All lanes : Anti-SHP2 antibody [Y478] ([ab32083](#)) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate

Lane 2 : PTPN11 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

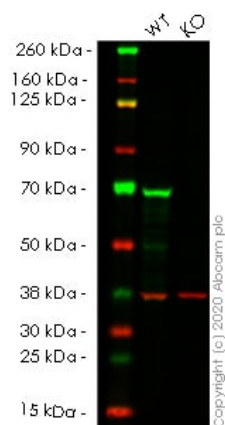
Predicted band size: 68 kDa

Observed band size: 68 kDa

Lanes 1- 2: Merged signal (red and green). Green - [ab32083](#) observed at 68 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) observed at 37 kDa.

[ab32083](#) was shown to react with SHP2 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line ab266450 (knockout cell lysate [ab257618](#)) was used. Wild-type HEK-293T and PTPN11 knockout HEK-293T 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.

ab32083 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.



Western blot - Human PTPN11 (SHP2) knockout HEK293T cell line (ab266450)

All lanes : Anti-SHP2 antibody [EPR17829-9] (**ab187040**) at 1/5000 dilution

Lane 1 : Wild-type HEK-293T cell lysate

Lane 2 : PTPN11 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 68 kDa

Observed band size: 68 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab187040** observed at 68 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab187040 was shown to react with SHP2 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line ab266450 (knockout cell lysate **ab257618**) was used. Wild-type HEK-293T and PTPN11 knockout HEK-293T 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.

ab187040 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 5000 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.

Mut	GAGCCGGAGGGCGGGAGGAACATGACATC--GGAGGTGAGGAGCCCCGAGGGGCCCGGC
WT	GAGCCGGAGGGCGGGAGGAACATGACATCGCGGAGGTGAGGAGCCCCGAGGGGCCCGGC

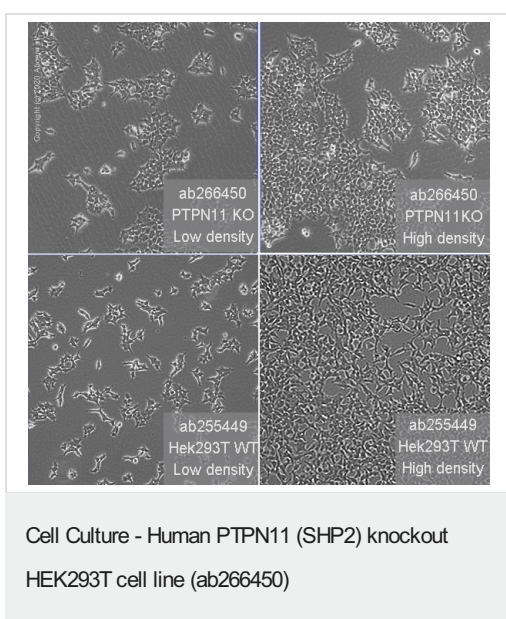
Sanger Sequencing - Human PTPN11 knockout
HEK293T cell line (ab266450)

Allele-1: 2 bp deletion in exon1

Mut	GAGCCGGAGGGCGGGAGGAACATGACATCGCGGAGGTGAGGAGCCCCGAGGGGCCCGGC
WT	GAGCCGGAGGGCGGGAGGAACATGACATCGCGGAGGTGAGGAGCCCCGAGGGGCCCGGC

Sanger Sequencing - Human PTPN11 knockout
HEK293T cell line (ab266450)

Allele-2: 1 bp insertion in exon 1.



Representative images PTPN11 knockout HEK293T cells, low and high confluency examples (top left and right respectively) and wild-type HEK293T cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using a EVOS M5000 microscope.

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