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Product datasheet

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

5 Images

Overview

Product name	Human 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)
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**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. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM (High Glucose) + 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 $2x10^4$ 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 $2x10^4$ 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.
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We will provide viable cells that proliferate on revival.

Properties

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
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

Function	Acts downstream of various receptor and cytoplasmic protein tyrosine kinases to participate in the signal transduction from the cell surface to the nucleus.
Tissue specificity	Widely expressed, with highest levels in heart, brain, and skeletal muscle.
Involvement in disease	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. 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. 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. 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.
Sequence similarities	Belongs to the protein-tyrosine phosphatase family. Non-receptor class 2 subfamily. Contains 2 SH2 domains. Contains 1 tyrosine-protein phosphatase domain.
Domain	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.
Post-translational modifications	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.
Cellular localization	Cytoplasm.

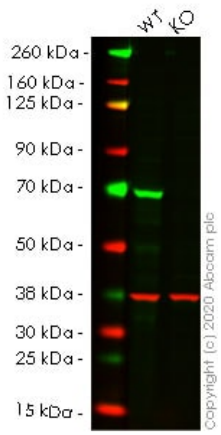
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab266450 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: 68 kDa.

Images



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

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.

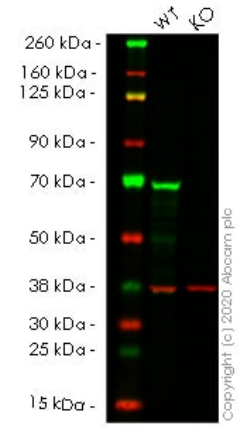
Performed under reducing conditions.

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.

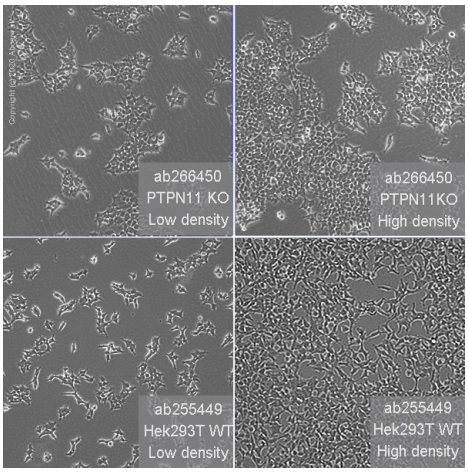
ab187040 was shown to react with SHP2 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line ab266450 (knockout cell lysate **ab257618**) was used. Wild-type HEK-293T and PTPN11 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for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

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

Allele-1: 2 bp deletion in exon1

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

Allele-2: 1 bp insertion in exon 1.



Cell Culture - Human PTPN11 (SHP2) knockout HEK293T cell line (ab266450)

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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