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Product datasheet

Human PDGFRB knockout SH-SY5Y cell line ab273749

3 Images

Overview

Product name	Human PDGFRB knockout SH-SY5Y cell line
Parental Cell Line	SHSY-5Y
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 5 bp deletion in exon 3
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	1
General notes	**Recommended control:** Human wild-type SHSY-5Y cell line (**ab275475**). 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:** 1:1 mixture of EMEM and 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 1-2x10^4 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. These cells grow as a mixture of floating and adherent cells. Remove media containing floating cells and recover cells by centrifugation, detach cells using standard methods, combine with floating cells and transfer to a new culture flask.

A guide seeding density of 1-2x10^4 cells/cm^2 is recommended.
Cells should be seeded at a density conducive to cell–cell communication to proliferate. If cells are seeded too sparsely, growth rate is reduced and cell death is high.
This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.

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	Bone marrow
Cell type	neuroblastoma
Disease	Neuroblastoma
Gender	Female
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	Receptor that binds specifically to PDGFB and PDGFD and has a tyrosine-protein kinase activity. Phosphorylates Tyr residues at the C-terminus of PTPN11 creating a binding site for the SH2 domain of GRB2.
Involvement in disease	Note=A chromosomal aberration involving PDGFRB is found in a form of chronic myelomonocytic leukemia (CMML). Translocation t(5;12)(q33;p13) with EVT6/TEL. It is characterized by abnormal clonal myeloid proliferation and by progression to acute myelogenous leukemia (AML). Note=A chromosomal aberration involving PDGFRB may be a cause of acute myelogenous leukemia. Translocation t(5;14)(q33;q32) with TRIP11. The fusion protein may be involved in clonal evolution of leukemia and eosinophilia. Note=A chromosomal aberration involving PDGFRB may be a cause of juvenile myelomonocytic leukemia. Translocation t(5;17)(q33;p11.2) with SPECC1. Defects in PDGFRB are a cause of myeloproliferative disorder chronic with eosinophilia (MPE) [MIM:131440]. A hematologic disorder characterized by malignant eosinophils proliferation. Note=A chromosomal aberration involving PDGFRB is found in many instances of myeloproliferative disorder chronic with eosinophilia. Translocation t(5;12) with ETV6 on chromosome 12 creating an PDGFRB-ETV6 fusion protein. Note=A chromosomal aberration involving PDGFRB may be the cause of a myeloproliferative disorder (MBD) associated with eosinophilia. Translocation t(1;5)(q23;q33) that forms a PDE4DIP-PDGFRB fusion protein.
Sequence similarities	Belongs to the protein kinase superfamily. Tyr protein kinase family. CSF-1/PDGF receptor subfamily. Contains 5 Ig-like C2-type (immunoglobulin-like) domains. Contains 1 protein kinase domain.

Post-translational modifications	Autophosphorylated. Dephosphorylated by PTPRJ at Tyr-751, Tyr-857, Tyr-1009 and Tyr-1021.
Cellular localization	Membrane.

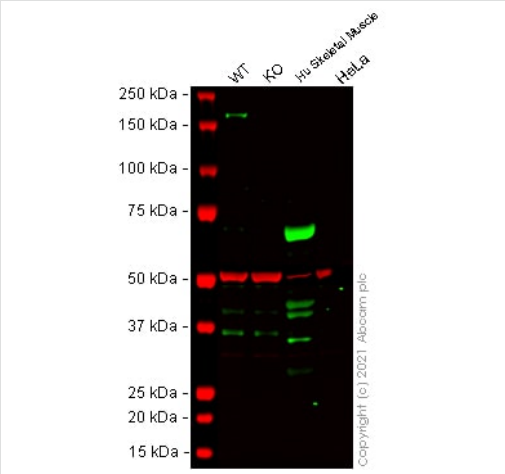
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab273749 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.

Images



Western blot - Human PDGFRB knockout SHSY-5Y cell line (ab273749)

All lanes : Anti-PDGFR beta antibody [42G12] (**ab69506**) at 1/1000 dilution

Lane 1 : Wild-type SH-SY5Y cell lysate
Lane 2 : PDGFRB knockout SH-SY5Y cell lysate
Lane 3 : Human Skeletal Muscle tissue lysate
Lane 4 : HeLa cell lysate

Lysates/proteins at 30 µg per lane.

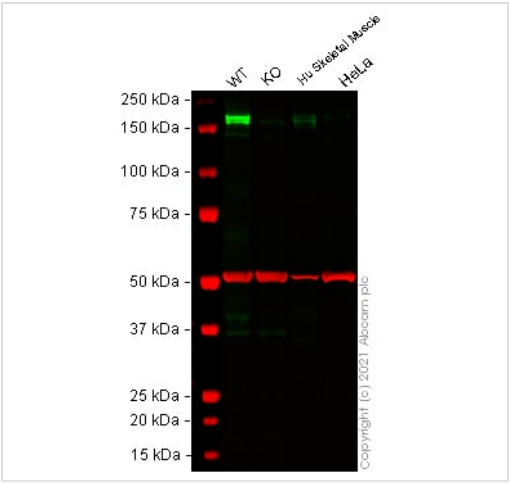
Performed under reducing conditions.

Observed band size: 170 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab69506** observed at 170 kDa. Red - loading control **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55 kDa.

ab69506 was shown to react with PDGFR beta in wild-type SH-SY5Y cells in Western blot with loss of signal observed in PDGFRB knockout cell line ab273749 (knockout cell lysate **ab275523**). Wild-type SH-SY5Y and PDGFRB knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab69506** and **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW)

preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Human PDGFRB knockout SHSY-5Y cell line (ab273749)

All lanes : Anti-PDGFR alpha + PDGFR beta antibody [Y92] - C-terminal (**ab32570**) at 1/5000 dilution

Lane 1 : Wild-type SH-SY5Y cell lysate
Lane 2 : PDGFR beta knockout SH-SY5Y cell lysate
Lane 3 : Human Skeletal Muscle tissue lysate
Lane 4 : HeLa cell lysate

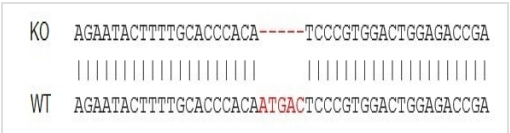
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Observed band size: 170 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab32570** observed at 170 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab32570 was shown to react with PDGFRB in wild-type SH-SY5Y cells in Western blot with loss of signal observed in PDGFRB knockout cell line ab273749 (knockout cell lysate **ab275523**). Wild-type SH-SY5Y and PDGFRB knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab32570** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 5000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Sanger Sequencing - Human PDGFRB knockout SHSY-5Y cell line (ab273749)

Allele-1: 5 bp deletion in exon 3

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