abcam

Product datasheet

Human PNP (Nucleoside phosphorylase) knockout HEK-293T cell line ab266158

画像数 3

製品の概要

製品名	Human PNP (Nucleoside phosphorylase) knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 2
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 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.

製品の特性

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
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

関連疾患	Defects in PNP are the cause of purine nucleoside phosphorylase deficiency (PNP deficiency) [MIM:613179]. It leads to a severe T-cell immunodeficiency with neurologic disorder in children.
配列類似性	Belongs to the PNP/MTAP phosphorylase family.
細胞内局在	Cytoplasm > cytoskeleton.

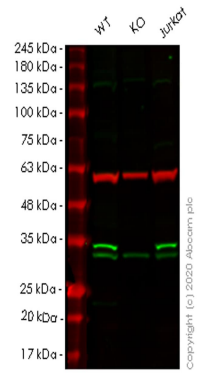
アプリケーション

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ab266158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: 32 kDa.

画像



Western blot - Human PNP knockout HEK293T cell line (ab266158)

All lanes : Anti-Nucleoside phosphorylase antibody [EPR5715] (**ab109447**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PNP knockout HeLa cell lysate
Lane 3 : Jurkat cell lysate

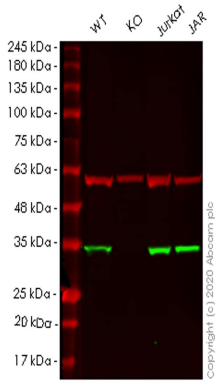
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 32 kDa
Observed band size: 31 kDa

Lanes 1-3: Merged signal (red and green). Green - **ab109447** observed at 31 kDa. Red - loading control **ab7291** observed at 50 kDa.

ab109447 Anti-Nucleoside phosphorylase antibody [EPR5715] was shown to specifically react with Nucleoside phosphorylase in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab266158 (knockout cell lysate **ab257594**) was used. Wild-type and Nucleoside phosphorylase knockout samples were subjected to SDS-PAGE. **ab109447** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) were incubated at room temperature for 2.5 hours at 1 in 1000 dilution and 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 PNP knockout HEK293T cell line (ab266158)

All lanes : Anti-Nucleoside phosphorylase antibody [EPR5714] (**ab109559**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PNP knockout HeLa cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : JAR cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 32 kDa
Observed band size: 31 kDa

Lanes 1-4: Merged signal (red and green). Green - **ab109559** observed at 31 kDa. Red - loading control **ab7291** observed at 50 kDa.

ab109559 Anti-Nucleoside phosphorylase antibody [EPR5714] was shown to specifically react with Nucleoside phosphorylase in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab266158 (knockout cell lysate **ab257594**) was used. Wild-type and Nucleoside phosphorylase knockout samples were subjected to SDS-PAGE. **ab109559** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) were incubated at room temperature for 2. 5 hours at 1 in 1000 dilution and 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 PNP knockout HEK293T cell line (ab266158)

Homozygous: 1 bp insertion in exon 2

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