Overview

**Product name**  
Human PNP (Nucleoside phosphorylase) knockout HEK293T 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)

**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-TMK1.

> **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 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 (Click here to view haemocytometer protocol) or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of 2x10⁴ cells/cm². This should allow for confluency within 48 hours. 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.

**Subculture guidelines:**

All seeding densities should be based on cell counts gained by established methods.
A guide seeding density of $2 \times 10^4$ cells/cm$^2$ is recommended for confluency (80-90% confluence) within 48 hours.

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.

Click here to view the Mammalian cell tissue culture protocol

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Properties

<table>
<thead>
<tr>
<th>Number of cells</th>
<th>1 x $10^6$ cells/vial, 1 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viability</td>
<td>~90%</td>
</tr>
<tr>
<td>Adherent/Suspension</td>
<td>Adherent</td>
</tr>
<tr>
<td>Tissue</td>
<td>Kidney</td>
</tr>
<tr>
<td>Cell type</td>
<td>epithelial</td>
</tr>
<tr>
<td>STR Analysis</td>
<td>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</td>
</tr>
<tr>
<td>Mycoplasma free</td>
<td>Yes</td>
</tr>
<tr>
<td>Storage instructions</td>
<td>Shipped on Dry Ice. Store in liquid nitrogen.</td>
</tr>
<tr>
<td>Storage buffer</td>
<td>Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether</td>
</tr>
<tr>
<td>Purity</td>
<td>Immunogen affinity purified</td>
</tr>
</tbody>
</table>

Target

<table>
<thead>
<tr>
<th>Involvement in disease</th>
<th>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.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence similarities</td>
<td>Belongs to the PNP/MTAP phosphorylase family.</td>
</tr>
<tr>
<td>Cellular localization</td>
<td>Cytoplasm &gt; cytoskeleton.</td>
</tr>
</tbody>
</table>

Applications

Our Abpromise guarantee covers the use of ab266158 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td></td>
<td>Use at an assay dependent concentration. Predicted molecular weight: 32 kDa.</td>
</tr>
</tbody>
</table>

Images
**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

*why is the actual band size different from the predicted?*

**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  *why is the actual band size different from the predicted?*

**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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