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Product datasheet

Human TMEM173 knockout THP-1 cell line ab270493

5 Images

Overview

Product name	Human TMEM173 knockout THP-1 cell line
Parental Cell Line	THP-1
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 1 insertion; Frameshift: 99.58%
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS), Western Blot (WB)
Tested applications	**Suitable for:** WB, Next Generation Sequencing
Biosafety level	1
General notes	**Recommended control:** Human wild-type THP-1 cell line (**ab271147**). 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:** RPMI + 10% FBS + 0.05 mM β-mercaptoethanol **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 2-4x10^5 cells/mL. 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. 5. THP-1 cells recover slowly from cryopreservation and therefore may not be ready for subculture for a number of days. Cells should be left as much as possible over this time and only subcultured when the cell density reaches 8x10^5 cells/mL. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods.

Cells should be seeded at 2-4x10^5 cells/mL and subcultured when they have reached 8x10^5 cells/mL. It is not recommended to allow the cell density to exceed 1x10^6 cells/mL.
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.
This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and 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	Suspension
Tissue	Blood
Cell type	acute monocytic leukemia
Disease	Acute Monocytic Leukemia
Gender	Male
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	Facilitator of innate immune signaling that promotes the production of type I interferon (IFN-alpha and IFN-beta). Innate immune response is triggered in response to non-CpG double-stranded DNA from viruses and bacteria delivered to the cytoplasm. Able to activate both NF-kappa-B and IRF3 transcription pathways to induce expression of type I interferon and exert a potent anti-viral state following expression. May be involved in translocon function, the translocon possibly being able to influence the induction of type I interferons. May be involved in transduction of apoptotic signals via its association with the major histocompatibility complex class II (MHC-II). Mediates death signaling via activation of the extracellular signal-regulated kinase (ERK) pathway.
Tissue specificity	Ubiquitously expressed.
Sequence similarities	Belongs to the TMEM173 family.
Post-translational modifications	Phosphorylated on tyrosine residues upon MHC-II aggregation (By similarity). Phosphorylated on Ser-358 by TBK1, leading to activation and production of IFN-beta. Ubiquitinated. 'Lys-63'-linked ubiquitination mediated by TRIM56 at Lys-150 promotes homodimerization and recruitment of the antiviral kinase TBK1 and subsequent production of IFN-beta. 'Lys-48'-linked polyubiquitination at Lys-150 occurring after viral infection is mediated by RNF5 and leads to proteasomal degradation.
Cellular localization	Endoplasmic reticulum membrane. Mitochondrion outer membrane. Cell membrane. Cytoplasm > perinuclear region. In response to double-stranded DNA stimulation, relocalizes to perinuclear region, where the kinase TBK1 is recruited.

Applications

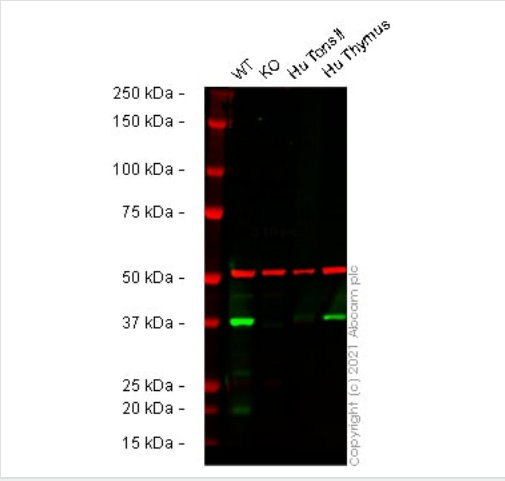
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab270493 in the following tested applications.

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: 42 kDa.
Next Generation Sequencing		Use at an assay dependent concentration.

Images

Next Generation Sequencing - Human TMEM173 knockout THP-1 cell line (ab270493)



Western blot - Human TMEM173 knockout THP-1 cell line (ab270493)

All lanes : Anti-STING antibody [EPR13130] (**ab181125**) at 1/1000 dilution

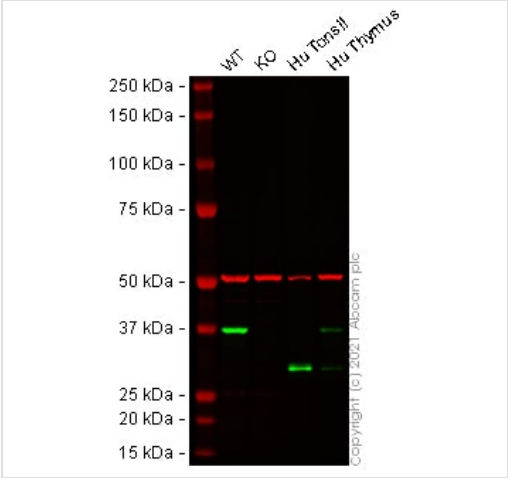
Lane 1 : Wild-type THP-1 cell lysate
Lane 2 : TMEM173 knockout THP-1 cell lysate
Lane 3 : Human Tonsil cell lysate
Lane 4 : Human Thymus cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 42 kDa
Observed band size: 37 kDa

False colour image of Western blot: Anti-STING antibody [EPR13130] staining at 1/1000 dilution, shown in green; loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) staining at 1/20000 dilution, shown in red. In Western blot, **ab181125** was shown to bind specifically to STING. A band was observed at 37 kDa in wild-type THP-1 cell lysates with no signal observed at this size in TMEM173 knockout cell line ab270493 (knockout cell lysate **ab270516**). To generate this image, wild-type and TMEM173 knockout THP-1 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Human TMEM173 knockout THP-1 cell line (ab270493)

All lanes : Anti-STING antibody [EPR13130-55] (**ab239074**) at 1/1000 dilution

Lane 3 : Human Tonsil tissue lysate
Lane 4 : Human Thymus tissue lysate

Lysates/proteins at 20 µg per lane.

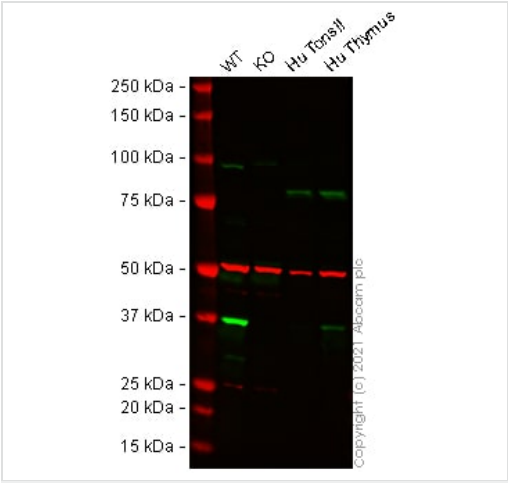
Performed under reducing conditions.

Predicted band size: 42 kDa
Observed band size: 37 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab239074** observed at 37 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab239074 was shown to react with TMEM173 in wild-type THP-1 cells in Western blot with loss of signal observed in TMEM173 knockout cell line ab270493 (TMEM173 knockout cell lysae **ab270516**). Wild-type THP-1 and TMEM173 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab239074** and **ab7291** (Mouse anti-Alpha Tubulin

[DM1A]) overnight at 4 °C at a 1 in 1000 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.



Western blot - Human TMEM173 knockout THP-1 cell line (ab270493)

All lanes : Anti-STING antibody [SP339] (**ab227705**) at 1/400 dilution

Lane 1 : Wild-type THP-1 cell lysate
Lane 2 : TMEM173 knockout THP-1 cell lysate
Lane 3 : Human Tonsil tissue lysate
Lane 4 : Human Thymus tissue lysate

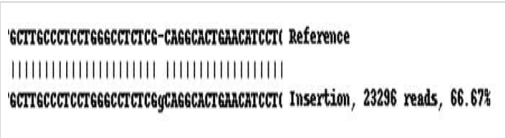
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 42 kDa
Observed band size: 37 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab227705** observed at 37 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab227705 was shown to react with TMEM173 in wild-type THP-1 cells in Western blot with loss of signal observed in TMEM173 knockout cell line ab270493 (TMEM173 knockout cell lysate **ab270516**). Wild-type THP-1 and TMEM173 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab227705** and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 400 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.



Next Generation Sequencing - Human TMEM173 knockout THP-1 cell line (ab270493)

Knockout achieved by CRISPR/Cas9; X = 1 bp insertion;
Frameshift = 99.58%

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