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Product datasheet

Human TNF knockout THP-1 cell line ab273761

5 Images

Overview

Product name	Human TNF knockout THP-1 cell line
Parental Cell Line	THP-1
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 52 bp deletion in exon 4
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB, Sandwich ELISA
Biosafety level	1
General notes	**Recommended control:** Human wild-type THP-1 cell line (**ab275477**). 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 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	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	Cytokine that binds to TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. It is mainly secreted by macrophages and can induce cell death of certain tumor cell lines. It is potent pyrogen causing fever by direct action or by stimulation of interleukin-1 secretion and is implicated in the induction of cachexia, Under certain conditions it can stimulate cell proliferation and induce cell differentiation.
Involvement in disease	Genetic variations in TNF are a cause of susceptibility psoriatic arthritis (PSORAS) [MIM:607507]. PSORAS is an inflammatory, seronegative arthritis associated with psoriasis. It is a heterogeneous disorder ranging from a mild, non-destructive disease to a severe, progressive, erosive arthropathy. Five types of psoriatic arthritis have been defined: asymmetrical oligoarthritis characterized by primary involvement of the small joints of the fingers or toes; asymmetrical arthritis which involves the joints of the extremities; symmetrical polyarthritis characterized by a rheumatoidlike pattern that can involve hands, wrists, ankles, and feet; arthritis mutilans, which is a rare but deforming and destructive condition; arthritis of the sacroiliac joints and spine (psoriatic spondylitis).
Sequence similarities	Belongs to the tumor necrosis factor family.
Post-translational modifications	The soluble form derives from the membrane form by proteolytic processing. The membrane form, but not the soluble form, is phosphorylated on serine residues. Dephosphorylation of the membrane form occurs by binding to soluble TNFRSF1A/TNFR1. O-glycosylated; glycans contain galactose, N-acetylgalactosamine and N-acetylneuraminic acid.
Cellular localization	Secreted and Cell membrane.

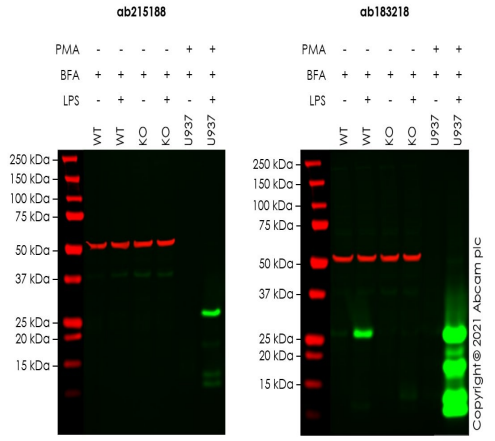
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab273761 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: 25 kDa.
Sandwich ELISA		Use at an assay dependent concentration.

Images



Western blot - Human TNF knockout THP-1 cell line (ab273761)

All lanes : Anti-TNF alpha antibody [EPR20972] (**ab215188**) at 1/1000 dilution

Lane 1 : Wild-type THP-1 control: Brefeldin A (5 ug/mL, 4 h) cell lysate
Lane 2 : Wild-type treated THP-1: LPS (100 ng/mL, 16 h), Brefeldin A (5 ug/mL, last 4 h) cell lysate
Lane 3 : TNF alpha knockout THP-1 control: Brefeldin A (5 ug/mL, 4 h) cell lysate
Lane 4 : TNF alpha knockout THP-1 treated: LPS (100 ng/mL, 16 h), Brefeldin A (5 ug/mL, last 4 h) cell lysate
Lane 5 : U937 control: PMA (10 mM, 2 days), Brefeldin A (5 ug/mL, last 4 h) cell lysate
Lane 6 : U937 treated: PMA (10 mM, 2 days), LPS (1 ug/mL, last 16 h), Brefeldin A (5 ug/mL, last 4 h) cell lysate

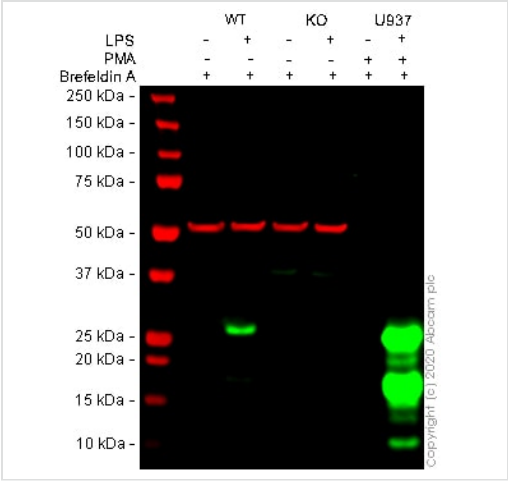
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 25 kDa
Observed band size: 27 kDa

This Western blot image is a comparison between **ab215188** and **ab183218** tested under the same conditions. While **ab215188** is suitable for WB for some samples, **ab183218** was found to be more sensitive. False colour image of Western blot: Anti-TNF alpha antibody [EPR20972] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining

at 1/20000 dilution, shown in red. In Western blot, **ab215188** was shown to bind specifically to TNF alpha. A band was observed at 27 kDa in treated U937 cell lysates with no signal observed at this size without treatment. No signal was observed in wild-type THP-1 cell lysates or in TNF knockout cell line ab273761 (knockout cell lysate **ab275507**) with **ab215188**. However, a band was observed at 27 kDa in treated wild-type THP-1 cell lysates with **ab183218**. To generate this image, 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 Â°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 TNF knockout THP-1 cell line (ab273761)

All lanes : Anti-TNF alpha antibody [EPR22598-212] (**ab255275**) at 1/1000 dilution

Lane 1 : Wild-type THP-1 Brefeldin A (**ab120299**) treated (5 µg/ml, 4 h) cell lysate
Lane 2 : Wild-type THP-1 LPS treated (100 ng/ml, 16 h) and Brefeldin A (**ab120299**) treated (5 µg/ml, 4 h) cell lysate
Lane 3 : TNF alpha knockout THP-1 Brefeldin A (**ab120299**) treated (5 µg/ml, 4 h) cell lysate
Lane 4 : TNF alpha knockout THP-1 LPS treated (100 ng/ml, 16 h) and Brefeldin A (**ab120299**) treated (5 µg/ml, 4 h) cell lysate
Lane 5 : U937 PMA treated (10 mM, 2 days) plus 16 h no treatment and Brefeldin A (**ab120299**) treated (5 µg/ml, 4 h) cell lysate
Lane 6 : U937 PMA treated (10 mM, 2 days) and LPS treated (1 µg/ml, 16 h) plus Brefeldin A (**ab120299**) treated (5 µg/ml, 4 h) cell lysate

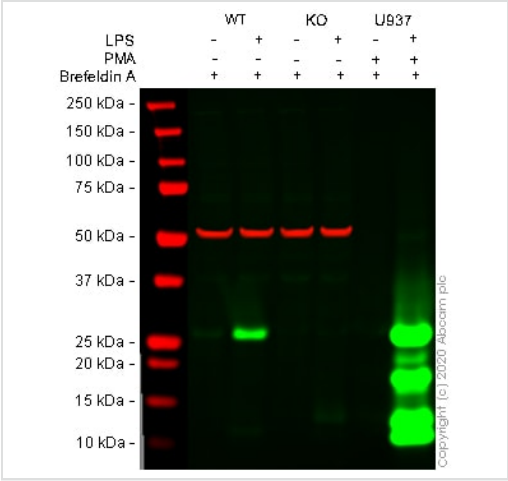
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 25 kDa
Observed band size: 26 kDa

Lanes 1 - 6: Merged signal (red and green). Green - **ab255275** observed at 26 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab255275 was shown to react with TNF alpha in THP-1 wild-type cells in Western blot with loss of signal observed in TNF knockout sample. Wild-type and TNF knockout THP-1 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab255275** 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 TNF knockout THP-1 cell line (ab273761)

All lanes : Anti-TNF alpha antibody [EPR19147] (**ab183218**) at 1/1000 dilution

Lane 1 : Wild-type THP-1 Brefeldin A (**ab120299**) treated (5 µg/ml, 4 h) cell lysate
Lane 2 : Wild-type THP-1 LPS treated (100 ng/ml, 16 h) and Brefeldin A (**ab120299**) treated (5 µg/ml, 4 h) cell lysate
Lane 3 : TNF alpha knockout THP-1 Brefeldin A (**ab120299**) treated (5 µg/ml, 4 h) cell lysate
Lane 4 : TNF alpha knockout THP-1 LPS treated (100 ng/ml, 16 h) and Brefeldin A (**ab120299**) treated (5 µg/ml, 4 h) cell lysate
Lane 5 : U937 PMA treated (10 mM, 2 days) plus 16 h no treatment and Brefeldin A (**ab120299**) treated (5 µg/ml, 4 h) cell lysate
Lane 6 : U937 PMA treated (10 mM, 2 days) and LPS treated (1 µg/ml, 16 h) plus Brefeldin A (**ab120299**) treated (5 µg/ml, 4 h) cell lysate

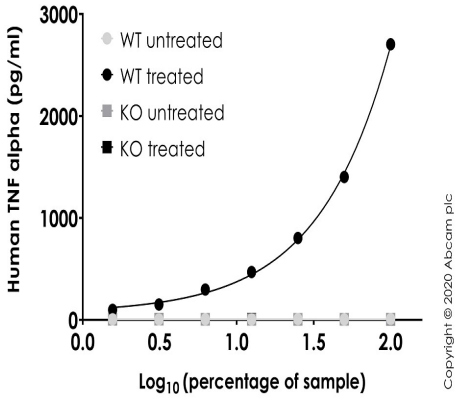
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 25 kDa
Observed band size: 26 kDa

Lanes 1 - 6: Merged signal (red and green). Green - **ab183218** observed at 26 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab183218 was shown to react with TNF alpha in THP-1 wild-type cells in Western blot with loss of signal observed in TNF knockout sample. Wild-type and TNF knockout THP-1 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab183218** 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.



Sandwich ELISA - Human TNF knockout THP-1 cell line (ab273761)

Human TNF alpha concentration was interpolated from the standard curve. Supernatants from cell culture samples were serially diluted and assessed by the Human TNF alpha ELISA kit (**ab181421**). Wild-type THP-1 and TNF alpha knockout THP-1 (ab273761) cells were assessed in duplicate (n=2). Cells were either treated with 100 ng/ml LPS for 16 h to induce expression of TNF alpha or not treated with LPS. Data are represented as the mean and error bars represent standard deviation."

Sanger Sequencing - Human TNF knockout THP-1 cell line (ab273761)

Homozygous: 52 bp deletion in exon 4

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