abcam

Product datasheet

Human TNF knockout THP-1 cell lysate ab275507

4 Images

Overview

Product name	Human TNF knockout THP-1 cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9. **Treatments:** Human TNF knockout THP-1 cell lysate - Brefeldin A (5 µg/ml, 6h) Human wild-type THP-1 cell lysate - Brefeldin A (5 µg/ml, 6h) Human TNF knockout THP-1 cell lysate - LPS (1 µg/ml, 16h) and Brefeldin A (5 µg/ml, during the last 6h of LPS treatment) Human wild-type THP-1 cell lysate - LPS (1 µg/ml, 16h) and Brefeldin A (5 µg/ml, during the last 6h of LPS treatment)
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)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
Notes	**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared. **User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage. Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. **See here for more information on knockout cell lysates.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.

It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

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**.

Tested applications **Suitable for:** WB

Properties

Storage instructions Store at -80°C. Please refer to protocols.

Components	1 kit
ab277319 - Human TNF knockout THP-1 cell lysate - Brefeldin A treated	1 x 100µg
ab277320 - Human TNF knockout THP-1 cell lysate - LPS + Brefeldin A treated	1 x 100µg
ab277492 - Human wild-type THP-1 cell lysate - Brefeldin A treated	1 x 100µg
ab277321 - Human wild-type THP-1 cell lysate - LPS + Brefeldin A treated	1 x 100µg

Cell type acute monocytic leukemia

Disease Acute Monocytic Leukemia

Gender Male

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.

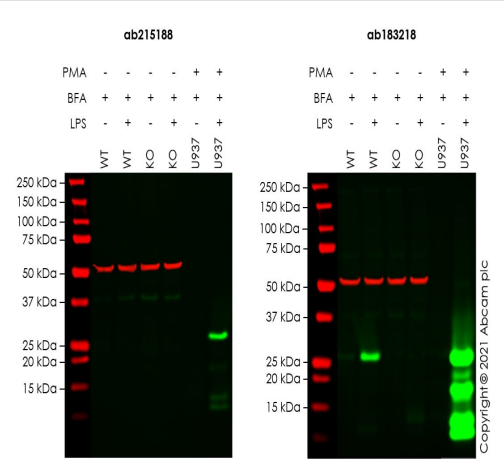
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab275507 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.

Images



Western blot - Human TNF knockout THP-1 cell lysate (ab275507)

Lane 1: Wild-type THP-1 control: Brefeldin A (5 µg/mL, 4 h) cell lysate 30 µg

Lane 2: Wild-type treated THP-1: LPS (100 ng/mL, 16 h), Brefeldin A (5 µg/mL, last 4 h) cell lysate 30 µg

Lane 3: TNF alpha knockout THP-1 control: Brefeldin A (5 µg/mL, 4 h) cell lysate 30 µg

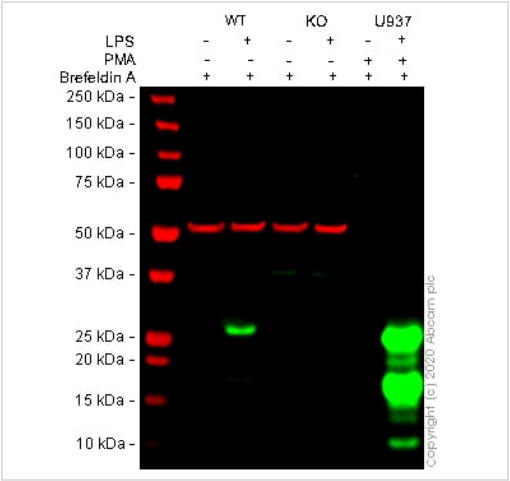
Lane 4: TNF alpha knockout THP-1 treated: LPS (100 ng/mL, 16 h), Brefeldin A (5 µg/mL, last 4 h) cell lysate 30 µg

Lane 5: U937 control: PMA (10 mM, 2 days), Brefeldin A (5 µg/mL, last 4 h) cell lysate 30 µg

Lane 6: U937 treated: PMA (10 mM, 2 days), LPS (1 µg/mL, last 16 h), Brefeldin A (5 µg/mL, last 4 h) cell lysate 30 µg

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 °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 lysate

(ab275507)

Lane 1: Wild-type THP-1 Brefeldin A (**ab120299**) treated (5 ug/ml, 4 h) cell lysate 30 ug

Lane 2: Wild-type THP-1 LPS treated (100 ng/ml, 16 h) and Brefeldin A (**ab120299**) treated (5 ug/ml, 4 h) cell lysate 30 ug

Lane 3: TNF alpha knockout THP-1 Brefeldin A (**ab120299**) treated (5 ug/ml, 4 h) cell lysate 30 ug

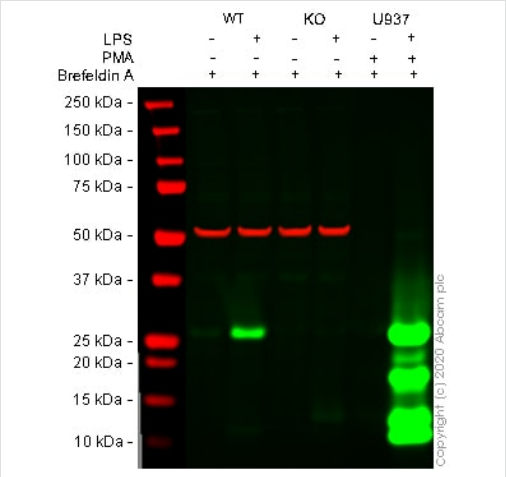
Lane 4: TNF alpha knockout THP-1 LPS treated (100 ng/ml, 16 h) and Brefeldin A (**ab120299**) treated (5 ug/ml, 4 h) cell lysate 30 ug

Lane 5: U937 PMA treated (10 mM, 2 days) plus 16 h no treatment and Brefeldin A (**ab120299**) treated (5 ug/ml, 4 h) cell lysate 30 ug

Lane 6: U937 PMA treated (10 mM, 2 days) and LPS treated (1 ug/ml, 16 h) plus Brefeldin A (**ab120299**) treated (5 ug/ml, 4 h) cell lysate 30 ug

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 55kDa.

ab255275 was shown to react with TNF alpha in wild-type THP-1 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 lysate

(ab275507)

Lane 1: Wild-type THP-1 Brefeldin A (**ab120299**) treated (5 ug/ml, 4 h) cell lysate 30 ug

Lane 2: Wild-type THP-1 LPS treated (100 ng/ml, 16 h) and Brefeldin A (**ab120299**) treated (5 ug/ml, 4 h) cell lysate 30 ug

Lane 3: TNF alpha knockout THP-1 Brefeldin A (**ab120299**) treated (5 ug/ml, 4 h) cell lysate 30 ug

Lane 4: TNF alpha knockout THP-1 LPS treated (100 ng/ml, 16 h) and Brefeldin A (**ab120299**) treated (5 ug/ml, 4 h) cell lysate 30 ug

Lane 5: U937 PMA treated (10 mM, 2 days) plus 16 h no treatment and Brefeldin A (**ab120299**) treated (5 ug/ml, 4 h) cell lysate 30 ug

Lane 6: U937 PMA treated (10 mM, 2 days) and LPS treated (1 ug/ml, 16 h) plus Brefeldin A (**ab120299**) treated (5 ug/ml, 4 h) cell lysate 30 ug

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 55kDa.

ab183218 was shown to react with TNF alpha in wild-type THP-1 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.

Sanger Sequencing - Human TNF knockout THP-1 cell lysate

Homozygous: 52 bp deletion in exon 4

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