abcam

Product datasheet

Human ANPEP (CD13) knockout THP-1 cell line ab273759

10 图像

概述

产品名称	人ANPEP (CD13) knockout THP-1 cell line
Parental Cell Line	THP-1
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 2 bp deletion in exon 2
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Sanger Sequencing, Western Blot (WB)
经测试应用	**适用于:** Flow Cyt, WB, ICC
Biosafety level	1
常规说明	**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.

性能

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
存放说明	Shipped on Dry Ice. Store in liquid nitrogen.
存储溶液	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

靶标

功能	Broad specificity aminopeptidase. Plays a role in the final digestion of peptides generated from hydrolysis of proteins by gastric and pancreatic proteases. May play a critical role in the pathogenesis of cholesterol gallstone disease. May be involved in the metabolism of regulatory peptides of diverse cell types including small intestinal and tubular epithelial cells, macrophages, granulocytes and synaptic membranes from the CNS. Found to cleave antigen peptides bound to major histocompatibility complex class II molecules of presenting cells and to degrade neurotransmitters at synaptic junctions. Is also implicated as a regulator of IL-8 bioavailability in the endometrium, and therefore may contribute to the regulation of angiogenesis. Is used as a marker for acute myeloid leukemia and plays a role in tumor invasion. In case of human coronavirus 229E (HCoV-229E) infection, serves as receptor for HCoV-229E spike glycoprotein. Mediates as well human cytomegalovirus (HCMV) infection.
组织特异性	Expressed in epithelial cells of the kidney, intestine, and respiratory tract; granulocytes, monocytes, fibroblasts, endothelial cells, cerebral pericytes at the blood-brain barrier, synaptic membranes of cells in the CNS. Also expressed in endometrial stromal cells, but not in the endometrial glandular cells. Found in the vasculature of tissues that undergo angiogenesis and in malignant gliomas and lymph node metastases from multiple tumor types but not in blood vessels of normal tissues. A soluble form has been found in plasma. It is found to be elevated in plasma and effusions of cancer patients.
序列相似性	Belongs to the peptidase M1 family.
结构域	Amino acids 260-353 are essential to mediate susceptibility to infection with HCoV-229E (in porcine/human chimeric studies) and more specifically amino acids 288-295 (mutagenesis

	studies).
翻译后修饰	Sulfated. N- and O-glycosylated. May undergo proteolysis and give rise to a soluble form.
细胞定位	Cell membrane. Cytoplasm > cytosol. A soluble form has also been detected.

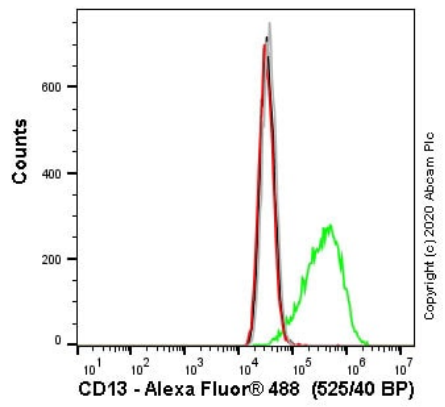
应用

The Abpromise guarantee **Abpromise™承诺保证使用ab273759于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration.
ICC		Use at an assay dependent concentration.

图片



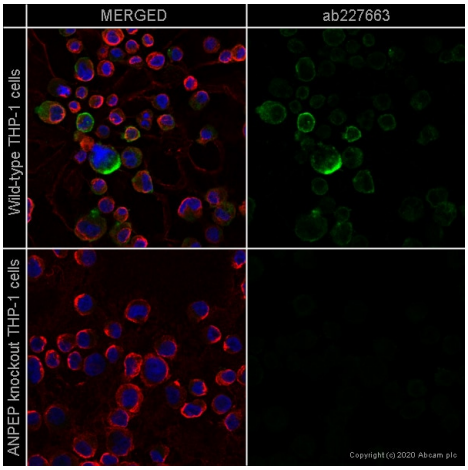
Flow Cytometry - Human ANPEP (CD13) knockout THP-1 cell line (ab273759)

Flow cytometry overlay histogram showing wild-type THP1 (green line) and ANPEP knockout THP1 cells (ab273759) stained with **ab20136** (red line). The cells were incubated in 1x PBS containing 10µg/ml human IgG and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (**ab20136**) (1x10^6 in 100µl at 1 µg/ml) for 30 min at 4°C.

The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150117**) was used at 1/2000 for 30 min at 4°C.

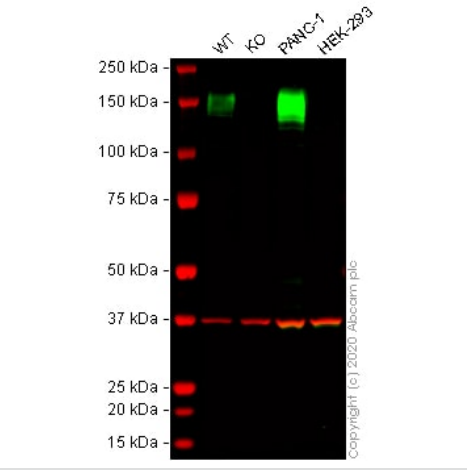
Isotype control antibody was mouse IgG2aκ (**ab18413**) used at the same concentration and conditions as the primary antibody (wild-type THP1 cells - black line; ANPEP knockout THP1 cells ab273759 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Immunocytochemistry/ Immunofluorescence - Human ANPEP (CD13) knockout THP-1 cell line (ab273759)

ab227663 staining CD13 in wild-type THP-1 cells (top panel) and ANPEP knockout THP-1 cells (bottom panel) (ab273759). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab227663** at 1µg/ml concentration and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Western blot - Human ANPEP (CD13) knockout THP-1 cell line (ab273759)

All lanes : Anti-CD13 antibody [SP187] (**ab227663**) at 1/400 dilution

Lane 1 : Wild-type THP-1 cell lysate
Lane 2 : ANPEP knockout THP-1 cell lysate
Lane 3 : PANC-1 cell lysate
Lane 4 : HEK-293 cell lysate

Lysates/proteins at 30 µg per lane.

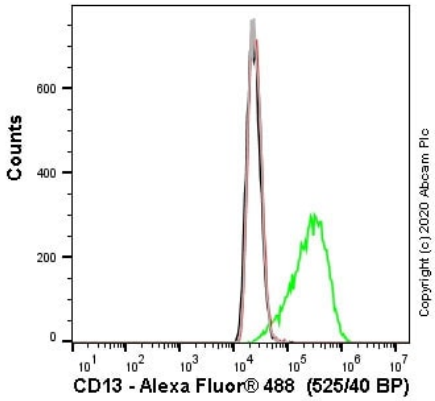
Performed under reducing conditions.

Observed band size: 160 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab227663** observed at 160 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab227663 was shown to react with CD13 in wild-type THP-1 cells in western blot with loss of signal observed in ANPEP knockout cell line ab273759 (knockout cell lysate **ab275505**). Wild-type and ANPEP knockout THP-1 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1%

Tween®) before incubation with **ab227663** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) 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 hour at room temperature before imaging.



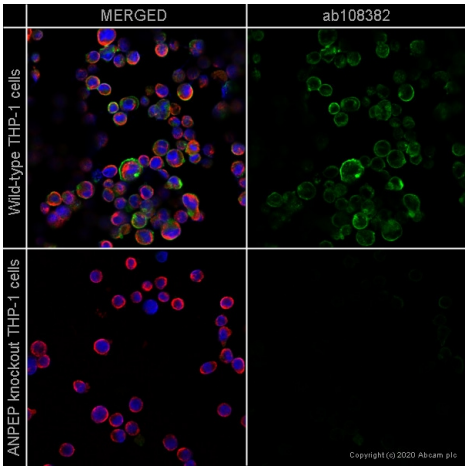
Flow Cytometry - Human ANPEP (CD13) knockout THP-1 cell line (ab273759)

Flow cytometry overlay histogram showing wild-type THP1 (green line) and ANPEP knockout THP1 cells (ab273759) stained with **ab7417** (red line). The cells were incubated in 1x PBS containing 10µg/ml human IgG and 10% normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody (**ab7417**) ($1x10^6$ in 100µl at 1 µg/ml) for 30 min at 4°C.

The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150117**) was used at 1/2000 for 30 min at 4°C.

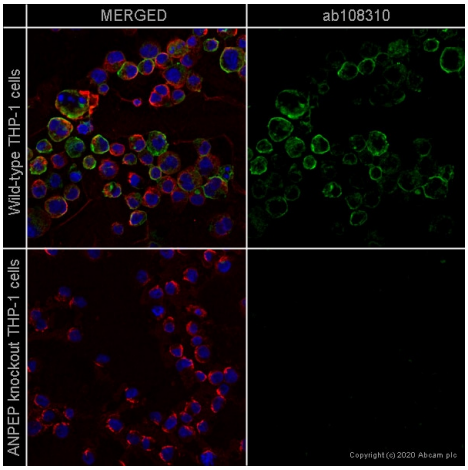
Isotype control antibody was mouse IgG1κ (**ab170190**) used at the same concentration and conditions as the primary antibody (wild-type THP1 cells - black line; ANPEP knockout THP1 cells ab273759 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



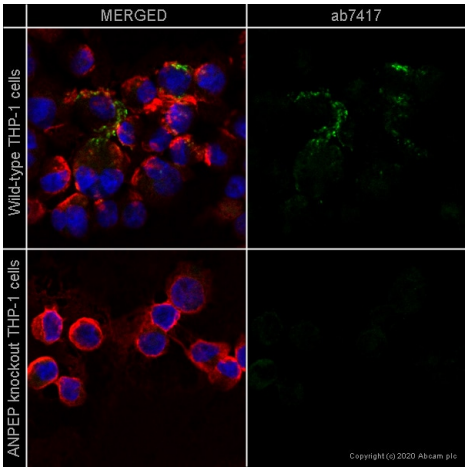
Immunocytochemistry/ Immunofluorescence - Human ANPEP (CD13) knockout THP-1 cell line (ab273759)

ab108382 staining CD13 in wild-type THP-1 cells (top panel) and ANPEP knockout THP-1 cells (bottom panel) (ab273759). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab108382** at 1/1000 dilution and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



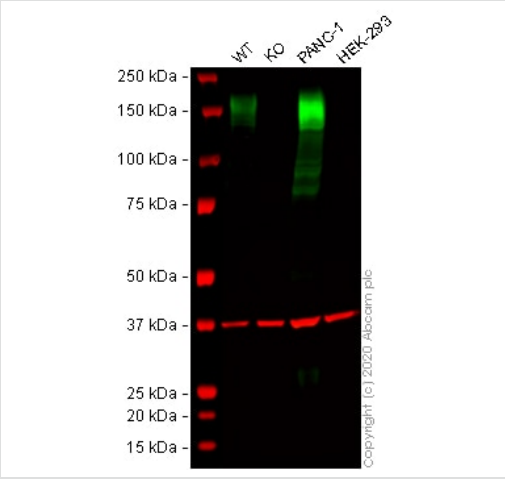
Immunocytochemistry/ Immunofluorescence - Human ANPEP (CD13) knockout THP-1 cell line (ab273759)

ab108310 staining CD13 in wild-type THP-1 cells (top panel) and ANPEP knockout THP-1 cells (bottom panel) (ab273759). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab108310** at 1/500 dilution and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Immunocytochemistry/ Immunofluorescence - Human ANPEP (CD13) knockout THP-1 cell line (ab273759)

ab7417 staining CD13 in wild-type THP-1 cells (top panel) and ANPEP knockout THP-1 cells (bottom panel) (ab273759). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab7417** at 2.5µg/ml concentration and **ab6046** (Rabbit polyclonal to beta Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to mouse IgG (Alexa Fluor® 488) (**ab150117**) at 2 µg/ml (shown in green) and a goat secondary antibody to rabbit IgG (Alexa Fluor® 594) (**ab150080**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Western blot - Human ANPEP (CD13) knockout THP-1 cell line (ab273759)

All lanes : Anti-CD13 antibody [EPR4059] (**ab108382**) at 1/1000 dilution

Lane 1 : Wild-type THP-1 cell lysate
Lane 2 : ANPEP knockout THP-1 cell lysate
Lane 3 : PANC-1 cell lysate
Lane 4 : HEK-293 cell lysate

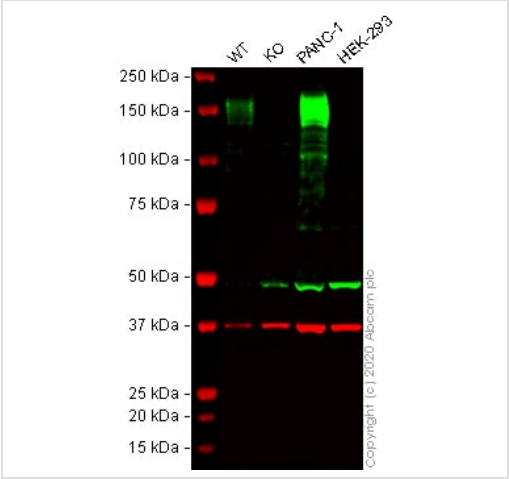
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Observed band size: 160 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab108382** observed at 160 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab108382 was shown to react with CD13 in wild-type THP-1 cells in western blot with loss of signal observed in ANPEP knockout cell line ab273759 (knockout cell lysate **ab275505**). Wild-type and ANPEP knockout THP-1 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab108382** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) 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 hour at room temperature before imaging.



Western blot - Human ANPEP (CD13) knockout THP-1 cell line (ab273759)

All lanes : Anti-CD13 antibody [EPR4058] (**ab108310**) at 1/1000 dilution

Lane 1 : Wild-type THP-1 cell lysate
Lane 2 : ANPEP knockout THP-1 cell lysate
Lane 3 : PANC-1 cell lysate
Lane 4 : HEK-293 cell lysate

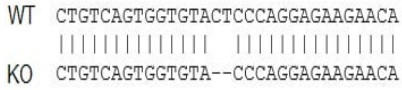
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Observed band size: 160 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab108310** observed at 160 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab108310 was shown to react with CD13 in wild-type THP-1 cells in western blot with loss of signal observed in ANPEP knockout cell line ab273759 (knockout cell lysate **ab275505**). Wild-type and ANPEP knockout THP-1 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with **ab108310** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) 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 hour at room temperature before imaging.



Sanger Sequencing - Human ANPEP (CD13) knockout THP-1 cell line (ab273759)

Homozygous: 2 bp deletion in exon 2

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