abcam

Product datasheet

Human ATG12 knockout THP-1 cell line ab277831

画像数 2

製品の概要

製品名	Human ATG12 knockout THP-1 cell line
Parental Cell Line	THP-1
Organism	Human
Passage number	<20
アプリケーション	**適用あり:** WB
Biosafety level	1
特記事項	**Recommended control:** Human wild-type THP-1 cell line (**ab281894**). 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.

製品の特性

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
保存方法	Shipped on Dry Ice. Store in liquid nitrogen.
バッファー	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

ターゲット情報

機能	Ubiquitin-like protein required for autophagy. Conjugated to ATG3 and ATG5.
組織特異性	Ubiquitous.
配列類似性	Belongs to the ATG12 family.
ドメイン	Shares weak sequence similarity with ubiquitin family, but contains an 'ubiquitin superfold' and the C-terminal Gly is required for isopeptide linkage.
細胞内局在	Cytoplasm.

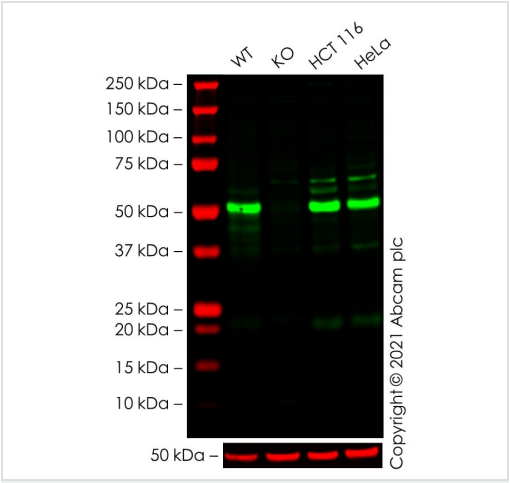
アプリケーション

The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab277831の使用に適用されます**

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration.

画像



Western blot - Human ATG12 knockout THP-1 cell line (ab277831)

All lanes : Anti-ATG12 antibody (**ab155589**) at 1/500 dilution

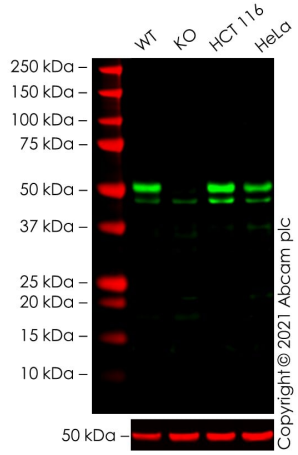
Lane 1 : Wild-type THP-1 cell lysate
Lane 2 : ATG12 knockout THP-1 cell lysate
Lane 3 : HCT 116 cell lysate
Lane 4 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 52 kDa

False colour image of Western blot: Anti-ATG12 antibody staining at 1/500 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab155589** was shown to bind specifically to ATG12. A band likely to be the unfunctional complex with ATG5 was observed at 52 kDa in wild-type THP-1 cell lysates with no signal observed at this size in Atg12 knockout cell line ab277831 (knockout cell lysate **ab278183**) - unconjugated functional form not observed at 15 kD. To generate this image, wild-type and Atg12 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 3% milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4Â°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 ATG12 knockout THP-1 cell line (ab277831)

All lanes : Anti-ATG12 antibody [EPR4800] (**ab109491**) at 1/1000 dilution

Lane 1 : Wild-type THP-1 cell lysate
Lane 2 : ATG12 knockout THP-1 cell lysate
Lane 3 : HCT 116 cell lysate
Lane 4 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 52 kDa

False colour image of Western blot: Anti-ATG12 antibody [EPR4800] 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, **ab109491** was shown to bind specifically to ATG12. A band likely to be the unfunctional complex with ATG5 was observed at 52 kDa in wild-type THP-1 cell lysates with no signal observed at this size in Atg12 knockout cell line ab277831 (knockout cell lysate **ab278183**) - unconjugated functional form not observed at 15 kD. To generate this image, wild-type and Atg12 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 3% milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4Â°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.

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