# abcam

### **Product datasheet**

## Human FCGR1A knockout THP-1 cell line ab275843

画像数4

製品の概要

製品名	Human FCGR1A knockout THP-1 cell line		
Parental Cell Line	THP-1		
Organism	Human		
Mutation description	Knockout achieved by CRISPR/Cas9; X = 1 bp deletion, 2 bp deletion; Frameshift: 99.99%		
Passage number	<20		
Knockout validation	Next Generation Sequencing (NGS)		
アプリケーション	適用あり: Next Generation Sequencing		
Biosafety level	1		
特記事項	<b>Recommended control:</b> Human wild-type THP-1 cell line ( <u>ab271147</u> ). 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.		
	<b>Cryopreservation cell medium:</b> 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		
	<b>Initial handling guidelines:</b> 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.		
	<ol> <li>Thaw the vial in 37°C water bath for approximately 1-2 minutes.</li> <li>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.</li> <li>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<sup>5</sup> cells/mL. Seeding density is given as a guide only and should be scaled to align with individual lab schedules.</li> <li>Incubate the culture at 37°C incubator with 5% CO<sub>2</sub>. Cultures should be monitored daily.</li> <li>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<sup>5</sup> cells/mL.</li> </ol>		
	Subculture guidelines:		

All seeding densities should be based on cell counts gained by established methods.

Cells should be seeded at  $2-4\times10^5$  cells/mL and subcultured when they have reached  $8\times10^5$  cells/mL. It is not recommended to allow the cell density to exceed  $1\times10^6$  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 <sup>6</sup> 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	High affinity receptor for the Fc region of immunoglobulins gamma. Functions in both innate and adaptive immune responses.
組織特異性	Monocyte/macrophage specific.
配列類似性	Belongs to the immunoglobulin superfamily. FCGR1 family. Contains 3 lg-like C2-type (immunoglobulin-like) domains.
翻訳後修飾	Phosphorylated on serine residues.
細胞内局在	Cell membrane. Stabilized at the cell membrane through interaction with FCER1G.

#### アプリケーション

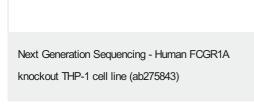
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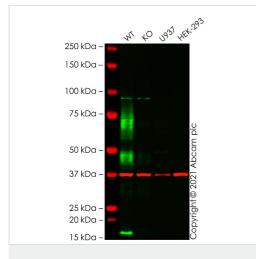
 The Abpromise guarantee
 Abpromise保証は、次のテスト済みアプリケーションにおけるab275843の使用に適用されます

 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
Next Generation Sequencing		Use at an assay dependent concentration.







Western blot - Human FCGR1A knockout THP-1 cell line (ab275843) 1 bp (allele 1) or 2 bp deletion (allele 2) after Asn 77 of the WT protein

All lanes : Anti-CD64 antibody [EPR4624] (<u>ab134073</u>) at 1/10000 dilution

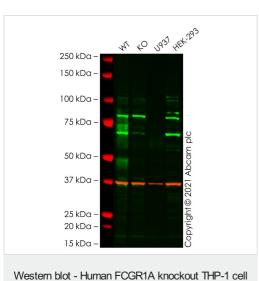
Lane 1 : Wild-type THP-1 cell lysate Lane 2 : FCGR1A knockout THP-1 cell lysate Lane 3 : U937 cell lysate Lane 4 : HEK-293 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 45-50 kDa

False colour image of Western blot: Anti-CD64 antibody [EPR4624] staining at 1/10000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab134073** was shown to bind specifically to CD64. A band was observed at 45-50/55-80 kDa in wild-type THP-1 cell lysates with no signal observed at this size in FCGR1A knockout cell line ab275843 (knockout cell lysate **ab275817**). To generate this image, wild-type and FCGR1A 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<sup>®</sup> 20 (TBS-T) 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<sup>®</sup> 800CW) preabsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed (<u>ab216776</u>) at 1/20000 dilution.



line (ab275843)

All lanes : Anti-CD64 antibody [EPR4623] (<u>ab109449</u>) at 1/1000 dilution

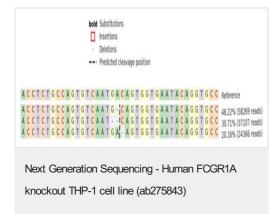
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Performed under reducing conditions.

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False colour image of Western blot: Anti-CD64 antibody [EPR4623] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab109449 was shown to bind specifically to CD64. A band was observed at 45-50/55-80 kDa in wild-type THP-1 cell lysates with no signal observed at this size in FCGR1A knockout cell line ab275843 (knockout cell lysate ab275817). To generate this image, wild-type and FCGR1A 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<sup>®</sup> 20 (TBS-T) 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<sup>®</sup> 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Knockout achieved by CRISPR/Cas9; X = 1 bp deletion, 2 bp deletion; Frameshift: 99.99%

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