

# Human RICTOR knockout A549 cell line ab277866

画像数 3

## 製品の概要

製品名	Human RICTOR knockout A549 cell line
Parental Cell Line	A549
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 43 bp deletion in exon 5
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
アプリケーション	適用あり: WB
Biosafety level	1
特記事項	<p><b>Recommended control:</b> Human wild-type A549 cell line (<a href="#">ab275463</a>). 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.</p> <p><b>Cryopreservation cell medium:</b> Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p><b>Culture medium:</b> F-12K + 10% FBS</p> <p><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.</p> <ol style="list-style-type: none"> <li>1. Thaw the vial in 37°C water bath for approximately 1-2 minutes.</li> <li>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.</li> <li>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 <math>2 \times 10^3</math>-<math>1 \times 10^4</math> cells/cm<sup>2</sup>. Seeding density is given as a guide only and should be scaled to align with individual lab schedules.</li> <li>4. Incubate the culture at 37°C incubator with 5% CO<sub>2</sub>. Cultures should be monitored daily.</li> </ol> <p><b>Subculture guidelines:</b></p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of <math>6 \times 10^4</math> cells/cm<sup>2</sup> is recommended.</p> <p>A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.</p>

Cells should be passaged when they have achieved 80-90% confluence.

Do not exceed  $7 \times 10^4$  cells/cm<sup>2</sup>.

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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	Adherent
Tissue	Lung
Cell type	epithelial
Disease	Carcinoma
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

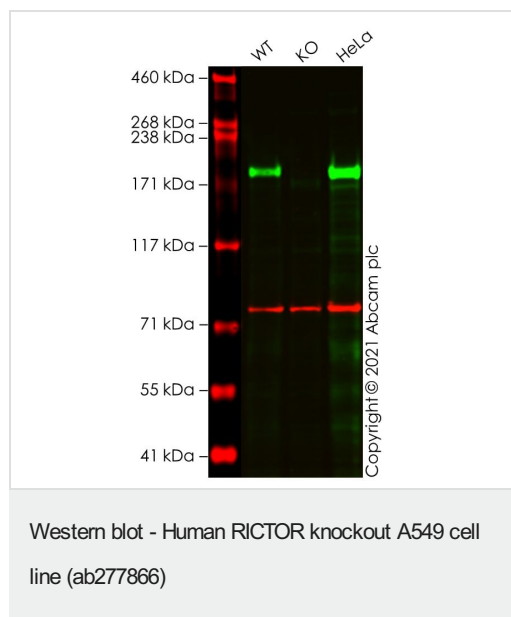
## ターゲット情報

機能	Subunit of mTORC2, which regulates cell growth and survival in response to hormonal signals. mTORC2 is activated by growth factors, but, in contrast to mTORC1, seems to be nutrient-insensitive. mTORC2 seems to function upstream of Rho GTPases to regulate the actin cytoskeleton, probably by activating one or more Rho-type guanine nucleotide exchange factors. mTORC2 promotes the serum-induced formation of stress-fibers or F-actin. mTORC2 plays a critical role in AKT1 'Ser-473' phosphorylation, which may facilitate the phosphorylation of the activation loop of AKT1 on 'Thr-308' by PDK1 which is a prerequisite for full activation. mTORC2 regulates the phosphorylation of SGK1 at 'Ser-422'. mTORC2 also modulates the phosphorylation of PRKCA on 'Ser-657'. Plays an essential role in embryonic growth and development.
配列類似性	Belongs to the RICTOR family.
翻訳後修飾	Phosphorylated by MTOR; when part of mTORC2. Phosphorylated at Thr-1135 by RPS6KB1; phosphorylation of RICTOR inhibits mTORC2 and AKT1 signaling.

## 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ab277866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. Detects a band of approximately 190 kDa (predicted molecular weight: 192 kDa).



**All lanes :** Anti-RICTOR antibody [EPR22008] ([ab219950](#)) at 1/1000 dilution

**Lane 1 :** Wild-type A549 cell lysate

**Lane 2 :** RICTOR knockout A549 cell lysate

**Lane 3 :** HeLa cell lysate

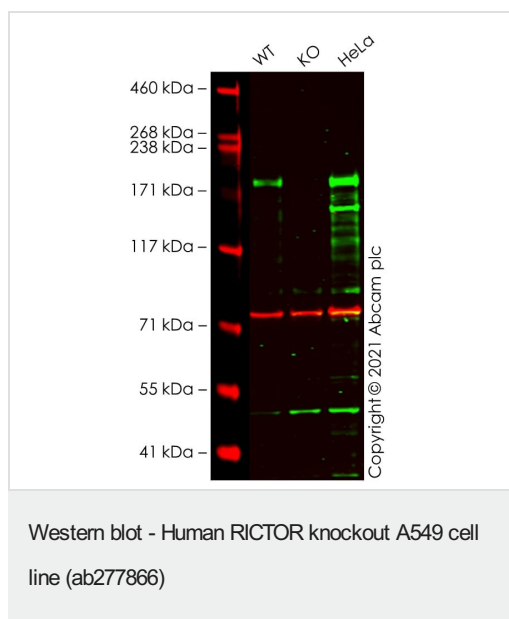
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 192 kDa

**Observed band size:** 190 kDa

False colour image of Western blot: Anti-RICTOR antibody [EPR22008] staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] ([ab238078](#)) loading control staining at 1/20000 dilution, shown in red. In Western blot, [ab219950](#) was shown to bind specifically to RICTOR. A band was observed at 190 kDa in wild-type A549 cell lysates with no signal observed at this size in RICTOR knockout cell line ab277866 (knockout cell lysate [ab288315](#)). To generate this image, wild-type and RICTOR knockout A549 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 ([ab216773](#)) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed ([ab216776](#)) at 1/20000 dilution.



**All lanes :** Anti-RICTOR antibody ([ab105469](#)) at 1 µg/ml

**Lane 1 :** Wild-type A549 cell lysate

**Lane 2 :** RICTOR knockout A549 cell lysate

**Lane 3 :** HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 192 kDa

**Observed band size:** 190 kDa

False colour image of Western blot: Anti-RICTOR antibody staining at 1 µg/ml, shown in green; Mouse anti-CANX [CANX/1543] ([ab238078](#)) loading control staining at 1/20000 dilution, shown in red. In Western blot, [ab105469](#) was shown to bind specifically to RICTOR. A band was observed at 190 kDa in wild-type A549 cell lysates with no signal observed at this size in RICTOR knockout cell line ab277866 (knockout cell lysate [ab288315](#)). To generate this image, wild-type and RICTOR knockout A549 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 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.

#### C4 - 43 bp deletion (main clone)

C4 GAAGTCGAGCA-----TTCTCCAGAGCTCTAAA  
|||||  
WT GAAGTCGAGCAGAGGCTACAGAGCTTTCATATCTCATCCAGAGCTCTCTTTCTCTCCAGAGCTCTAAA

43 bp deletion in exon 5

Sanger Sequencing - Human RICTOR knockout

A549 cell line (ab277866)

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