abcam

Product datasheet

Human ATF3 knockout A549 cell line ab266955

6 图像

概述

Parental Cell Line A549
Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, 14 bp deletion in exon 2 and 1 bp deletion in exon 2

and 4 bp deletion in exon 2

Passage number <20

Knockout validation Sanger Sequencing, Western Blot (WB)

2

经测试应用 适用于: WB

Biosafety level

常规说明 Recommended control: Human wild-type A549 cell line (<u>ab255450</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.

Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.

Culture medium: F-12K + 10% FBS

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 2x10³-1x10⁴ cells/cm². 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% CO₂. Cultures should be monitored daily.

Subculture guidelines:

All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $6x10^4$ cells/cm² is recommended.

A partial media change 24 hours prior to subculture may be helpful to encourage growth, if

1

required.

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

Do not exceed 7x10⁴ cells/cm².

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 <u>limited use license</u> and <u>patent pages</u>.

We will provide viable cells that proliferate on revival.

性能

Number of cells 1 x 10⁶ cells/vial. 1 mL

Adherent /Suspension Adherent

Tissue Lung

Cell type epithelial

Disease Carcinoma

Gender Male

STR Analysis Amelogenin X,Y D5S818: 11 D13S317: 11 D7S820: 8, 11 D16S539: 11, 12 wA: 14 TH01:

8,9.3 TPOX: 8,11 CSF1PO: 10, 12

Antibiotic resistance Puromycin 1.00µg/ml

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 This protein binds the cAMP response element (CRE) (consensus: 5'-GTGACGT[AC][AG]-3'), a

sequence present in many viral and cellular promoters. Represses transcription from promoters with ATF sites. It may repress transcription by stabilizing the binding of inhibitory cofactors at the promoter. Isoform 2 activates transcription presumably by sequestering inhibitory cofactors away

from the promoters.

序列相似性 Belongs to the bZIP family. ATF subfamily.

Contains 1 bZIP domain.

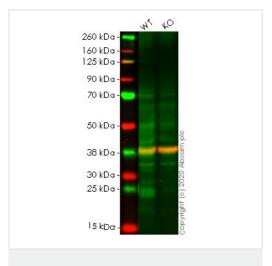
细胞定位 Nucleus.

应用

The Abpromise guarantee Abpromise™承诺保证使用ab266955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
WB		Use at an assay dependent concentration. Predicted molecular weight: 21 kDa.



Western blot - Human ATF3 knockout A549 cell line (ab266955)

All lanes : Anti-ATF3 antibody [EPR22610-19] - ChIP Grade (ab254268) at 1/1000 dilution

Lane 1: Wild-type A549 cell lysate

Lane 2: ATF3 knockout A549 cell lysate

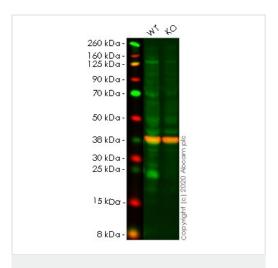
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 21 kDa **Observed band size:** 21 kDa

Lanes 1-2: Merged signal (red and green). Green - <u>ab254268</u> observed at 21 kDa. Red - loading control <u>ab8245</u> observed at 37 kDa.

ab254268 Recombinant Anti-ATF3 antibody [EPR22610-19] was shown to specifically react with ATF3 in wild-type A549 cells. Loss of signal was observed when knockout cell line ab266955 (knockout cell lysate ab257075) was used. Wild-type and ATF3 knockout samples were subjected to SDS-PAGE. ab254268 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human ATF3 knockout A549 cell line (ab266955)

All lanes : Anti-ATF3 antibody [EPR19488] - ChIP Grade (ab207434) at 1/1000 dilution

Lane 1: Wild-type A549 cell lysate

Lane 2: ATF3 knockout A549 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 21 kDa **Observed band size:** 21 kDa

Lanes 1-2: Merged signal (red and green). Green - <u>ab207434</u> observed at 21 kDa. Red - loading control <u>ab8245</u> observed at 37 kDa.

<u>ab207434</u> Anti-ATF3 antibody [EPR19488] - ChIP Grade was shown to specifically react with ATF3 in wild-type A549 cells. Loss of signal was observed when knockout cell line ab266955 (knockout cell lysate <u>ab257075</u>) was used. Wild-type and ATF3 knockout samples were subjected to SDS-PAGE. <u>ab207434</u> and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) were incubated overnight at 4°C at 1 in 1000 and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Allele-1: 14 bp deletion in exon2

Mut GCTAACCTGACGCCCTTTGTCAAGGAA----TGAGGTTTGCCATCCAGAACAAGCACCTC

GCTAACCTGACGCCCTTTGTCAAGGAAGAGCTGAGGTTTGCCATCCAGAACAAGCACCTC

Allele-2: 4 bp deletion in exon 2.

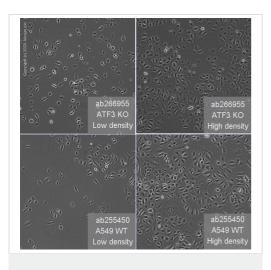
Sanger Sequencing - Human ATF3 knockout A549 cell line (ab266955)

Mut GCTAACCTGACGCCCTTTGTCAAGGAA-AGCTGAGGTTTGCCATCCAGAACAAGCACCTC

WT GCTAACCTGACGCCCTTTGTCAAGGAAGAGCTGAGGTTTGCCATCCAGAACAAGCACCTC

Allele-3: 1 bp deletion in exon 2.

Sanger Sequencing - Human ATF3 knockout A549 cell line (ab266955)



confluency examples (top left and right respectively) and wild-type A549 cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using a EVOS XL Core microscope.

Representative images of ATF3 knockout A549 cells, low and high

Cell Culture - Human ATF3 knockout A549 cell line (ab266955)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery

- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.cn/abpromise or contact our technical team.

Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors