abcam

Product datasheet

Human HLA-E (HLA E) knockout HEK-293T cell line ab267231

5 图像

概述

Parental Cell Line HEK293T
Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon 3 and 1 bp insertion in exon 3

and 8 bp deletion in exon 3

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 HEK293T cell line (<u>ab255449</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: DMEM (High Glucose) + 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 2x10⁴ 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 $2x10^4$ 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.

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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 Kidney
Cell type epithelial

STR Analysis Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01:

7, 9.3 TPOX: 11 CSF1PO: 11, 12

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

相关性 HLA E belongs to the HLA class I heavy chain paralogues. This class I molecule is a heterodimer

consisting of a heavy chain and a light chain (beta-2 microglobulin). The heavy chain is anchored in the membrane. HLA E binds a restricted subset of peptides derived from the leader peptides

of other class I molecules.

细胞定位 Membrane; Single-pass type I membrane protein

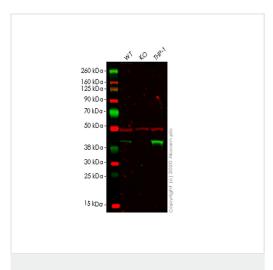
应用

The Abpromise guarantee Abpromise™承诺保证使用ab267231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: 40 kDa.

图片



Western blot - Human HLA-E (HLA E) knockout HEK-293T cell line (ab267231)

All lanes : Anti-HLA E antibody [MEM-E/02] (<u>ab2216</u>) at 1/500 dilution

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: HLA-E knockout HEK-293T cell lysate

Lane 3: THP-1 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 40 kDa Observed band size: 40 kDa

Lanes 1-3: Merged signal (red and green). Green - <u>ab2216</u> observed at 40 kDa. Red - loading control <u>ab52901</u> observed at kDa.

ab2216 Anti-HLA E antibody [MEM-E/02] was shown to specifically react with HLA E in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab267231 (knockout cell lysate ab258454) was used. Wild-type and HLA E knockout samples were subjected to SDS-PAGE. ab2216 and Anti-beta Tubulin [EP1331Y] - Microtubule Marker (ab52901) were incubated overnight at 4°C at 1 in 500 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 680RD) preadsorbed (ab216777) and Goat anti-Mouse lgG H&L (IRDye® 800CW) preadsorbed (ab216772) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

TATTTGTGGAGCCACTCCAC------CTTCCAGGTAGGCTCTCTGGTGCTCCGCCTCA TATTTGTGGAGCCACTCCACGCATGTGTCTTCCAGGTAGGCTCTCTGGTGCTCCGCCTCA WT Sanger Sequencing - Human HLA-E knockout HEK293T cell line (ab267231)

Allele-1: 8 bp deletion in exon3

Mut TATTTGTGGAGCCACTCCAC-CATGTGTCTTCCAGGTAGGCTCTCTGGTGCTCCGCCTCA TATTTGTGGAGCCACTCCACGCATGTGTCTTCCAGGTAGGCTCTCTGGTGCTCCGCCTCA

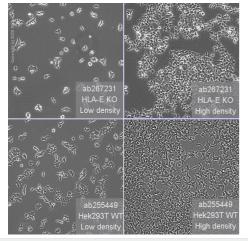
Allele-2: 1 bp deletion in exon 3.

Sanger Sequencing - Human HLA-E knockout HEK293T cell line (ab267231)

TATTTGTGGAGCCACTCCAC<mark>C</mark>GCATGTGTCTTCCAGGTAGGCTCTCTGGTGCTCCGCCTC Mut WT TATTIGTGGAGCCACTCCAC GCATGTGTCTTCCAGGTAGGCTCTCTGGTGCTCCGCCTC Allele-3: 1 bp insertion in exon 3.

Representative images of HLA-E knockout HEK293T cells, low and high confluency examples (top left and right respectively) and wildtype HEK293T cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using an EVOS M5000

Sanger Sequencing - Human HLA-E knockout HEK293T cell line (ab267231)



Cell Culture - Human HLA-E (HLA E) knockout

HEK293T cell line (ab267231)

microscope.

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