abcam

Product datasheet

# Human B2M (beta 2 Microglobulin) knockout HEK-293T cell line ab266828

5 Images

## Overview

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| **Product name** | Human B2M (beta 2 Microglobulin) knockout HEK-293T cell line |
| **Parental Cell Line** | HEK293T |
| **Organism** | Human |
| **Mutation description** | Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 1 |
| **Passage number** | <20 |
| **Knockout validation** | Sanger Sequencing |
| **Tested applications** | **Suitable for:** Sanger Sequencing, Cell Culture, WB |
| **Biosafety level** | 2 |
| **General notes** | **Recommended control:** Human wild-type HEK293T cell line (**ab255449**). 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.<br><br>**Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.<br><br>**Culture medium:** DMEM (High Glucose) + 10% FBS<br><br>**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.<br><br>1. Thaw the vial in 37°C water bath for approximately 1-2 minutes.<br>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.<br>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 $2x10^4$ cells/$cm^2$. Seeding density is given as a guide only and should be scaled to align with individual lab schedules.<br>4. Incubate the culture at 37°C incubator with 5% $CO_2$. Cultures should be monitored daily.<br><br>**Subculture guidelines:**<br><br>All seeding densities should be based on cell counts gained by established methods.<br>A guide seeding density of $2x10^4$ cells/$cm^2$ is recommended. |

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.
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## Properties

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| **Number of cells** | 1 x $10^6$ cells/vial, 1 mL |
| **Viability** | ~80% |
| **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 |
| **Antibiotic resistance** | Puromycin 1.00µg/ml |
| **Mycoplasma free** | Yes |
| **Storage instructions** | Shipped on Dry Ice. Store in liquid nitrogen. |
| **Storage buffer** | Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether |

## Target

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| **Function** | Component of the class I major histocompatibility complex (MHC). Involved in the presentation of peptide antigens to the immune system. |
| **Involvement in disease** | Defects in B2M are the cause of hypercatabolic hypoproteinemia (HYCATHYP) [MIM:241600]. Affected individuals show marked reduction in serum concentrations of immunoglobulin and albumin, probably due to rapid degradation.<br>Note=Beta-2-microglobulin may adopt the fibrillar configuration of amyloid in certain pathologic states. The capacity to assemble into amyloid fibrils is concentration dependent. Persistently high beta(2)-microglobulin serum levels lead to amyloidosis in patients on long-term hemodialysis. |
| **Sequence similarities** | Belongs to the beta-2-microglobulin family.<br>Contains 1 Ig-like C1-type (immunoglobulin-like) domain. |
| **Post-translational modifications** | Glycation of Ile-21 is observed in long-term hemodialysis patients. |
| **Cellular localization** | Secreted. Detected in serum and urine. |

## Applications

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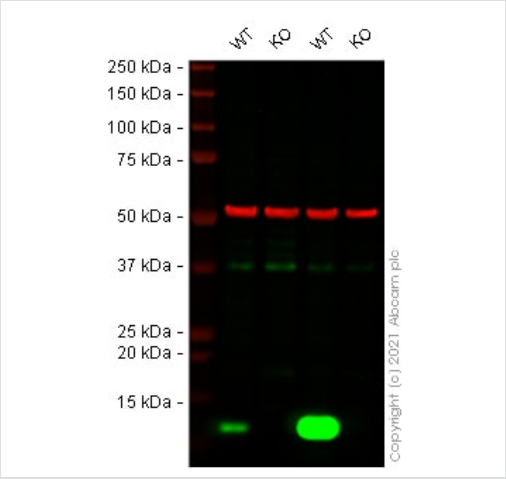
| **The Abpromise guarantee** | Our **Abpromise guarantee** covers the use of ab266828 in the following tested applications. |

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application | Abreviews | Notes |
|---|---|---|
| **Sanger Sequencing** | | Use at an assay dependent concentration. |
| **Cell Culture** | | Use at an assay dependent concentration. |
| **WB** | | Use at an assay dependent concentration. |

## Images




Western blot - Human B2M (beta 2 Microglobulin) knockout HEK-293T cell line (ab266828)

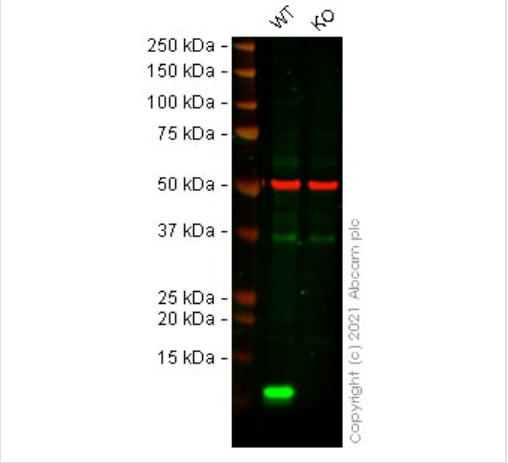
**All lanes :** Anti-beta 2 Microglobulin antibody [EP2978Y] (**ab75853**) at 1/5000 dilution

**Lane 1 :** Wild-type HEK-293T cell lysate
**Lane 2 :** B2M knockout HEK-293T cell lysate
**Lane 3 :** Wild-type A431 cell lysate
**Lane 4 :** B2M knockout A431 cell lysate

Performed under reducing conditions.

**Observed band size:** 12 kDa

False colour image of Western blot: Anti-beta 2 Microglobulin antibody [EP2978Y] staining at 1/5000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab75853** was shown to bind specifically to beta 2 Microglobulin. A band was observed at 12 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in B2M knockout cell line ab266828 (knockout cell lysate **ab256845**). To generate this image, wild-type and B2M knockout HEK-293T cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution 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.




Western blot - Human B2M (beta 2 Microglobulin) knockout HEK-293T cell line (ab266828)

**All lanes :** Anti-beta 2 Microglobulin antibody [EPR21752-214] (**ab218230**) at 1/1000 dilution

**Lane 1 :** Wild-type HEK-293T cell lysate
**Lane 2 :** B2M knockout HEK-293T cell lysate
**Lane 3 :** Wild-type A431 cell lysate
**Lane 4 :** B2M knockout A431 cell lysate

Performed under reducing conditions.

**Observed band size:** 12 kDa

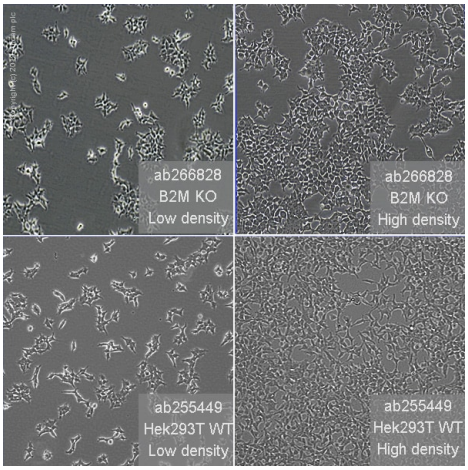
False colour image of Western blot: Anti-beta 2 Microglobulin antibody [EPR21752-214] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab218230** was shown to bind specifically to beta 2 Microglobulin. A band was observed at 12 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in B2M knockout cell line ab266828 (knockout cell lysate **ab256845**). To generate this image, wild-type and B2M knockout HEK-293T cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution 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.

Sanger Sequencing - Human B2M (beta 2 Microglobulin) knockout HEK-293T cell line (ab266828)

Sequencing chromatogram displaying sequence edit in exon 1


Sanger Sequencing - Human B2M knockout HEK293T cell line (ab266828)

Homozygous: 1 bp insertion in exon 1




Cell Culture - Human B2M (beta 2 Microglobulin) knockout HEK293T cell line (ab266828)

Representative images of B2M knockout HEK293T cells, low and high confluency examples (top left and right respectively) and wild-type HEK293T 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.



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