

### Human E2F4 knockout HEK-293T cell line ab266119

4 Images

#### Overview

<b>Product name</b>	Human E2F4 knockout HEK-293T cell line
<b>Parental Cell Line</b>	HEK293T
<b>Organism</b>	Human
<b>Mutation description</b>	Knockout achieved by using CRISPR/Cas9, 4 bp deletion in exon 2 and Insertion of the selection cassette in exon 2
<b>Passage number</b>	<20
<b>Knockout validation</b>	Sanger Sequencing, Western Blot (WB)
<b>Tested applications</b>	<b>Suitable for:</b> WB
<b>Biosafety level</b>	2
<b>General notes</b>	<p><b>Recommended control:</b> Human wild-type HEK293T cell line (<a href="#">ab255449</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> DMEM (High Glucose) + 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 10^4</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>2 \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</p>

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.

## Properties

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<b>Number of cells</b>	1 x 10 <sup>6</sup> cells/vial, 1 mL
<b>Adherent /Suspension</b>	Adherent
<b>Tissue</b>	Kidney
<b>Cell type</b>	epithelial
<b>STR Analysis</b>	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
<b>Mycoplasma free</b>	Yes
<b>Storage instructions</b>	Shipped on Dry Ice. Store in liquid nitrogen.
<b>Storage buffer</b>	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

## Target

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<b>Function</b>	Transcription activator that binds DNA cooperatively with DP proteins through the E2 recognition site, 5'-TTTC[CG]CGC-3' found in the promoter region of a number of genes whose products are involved in cell cycle regulation or in DNA replication. The DRTF1/E2F complex functions in the control of cell-cycle progression from G1 to S phase. E2F-4 binds with high affinity to RBL1 and RBL2. In some instances, can also bind RB protein.
<b>Tissue specificity</b>	Found in all tissue examined including heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas.
<b>Sequence similarities</b>	Belongs to the E2F/DP family.
<b>Developmental stage</b>	Present in the growth-arrested state, its abundance does not change significantly as cells move into and through the cell cycle.
<b>Post-translational modifications</b>	Differentially phosphorylated in vivo.
<b>Cellular localization</b>	Nucleus.

## Applications

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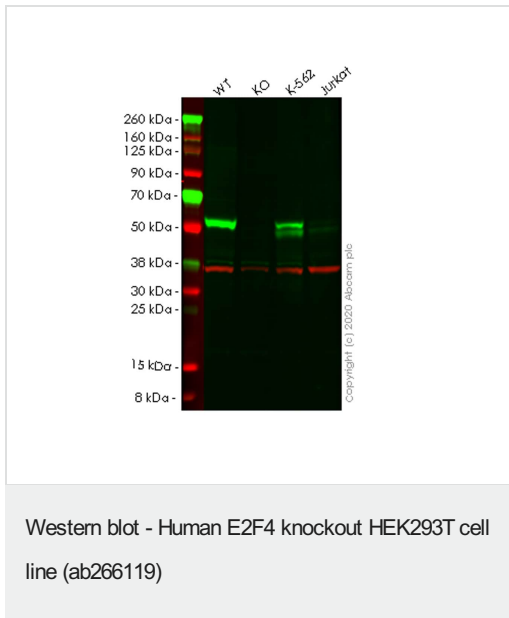
**The Abpromise guarantee** Our [Abpromise guarantee](#) covers the use of ab266119 in the following tested applications.

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

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 44 kDa.

Application	Abreviews	Notes

**Images**



**All lanes** : Anti-E2F4 antibody [EPR8259] ([ab150360](#)) at 1/1000 dilution

- Lane 1** : Wild-type HEK293T cell lysate
- Lane 2** : E2F4 knockout HEK293T cell lysate
- Lane 3** : K-562 cell lysate
- Lane 4** : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

**Secondary**

**All lanes** : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed ([ab216773](#)) at 1/10000 dilution

**Predicted band size:** 44 kDa  
**Observed band size:** 60 kDa

**Lanes 1-4:** Merged signal (red and green). Green - [ab150360](#) observed at 60 kDa. Red - loading control [ab8245](#) observed at 36 kDa.

[ab150360](#) Anti-E2F4 antibody [EPR8259] was shown to specifically react with E2F4 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266119 (knockout cell lysate [ab257932](#)) was used. Wild-type and E2F4 knockout samples were subjected to SDS-PAGE. [ab150360](#) and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) were incubated at room temperature for 2.5 hours at 1 in 1000 dilution 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.

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Mut  CTAATCGAGAAAAAGTCCAAGAACAGCA- - - GT GGAAGT GAGT GGCAT AGT GGGAGGG
      |||
WT   CTAATCGAGAAAAAGTCCAAGAACAGCATCCAGT GGAAGT GAGT GGCAT AGT GGGAGGG

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Allele-1: 4 bp deletion in exon2

Sanger Sequencing - Human E2F4 knockout

HEK293T cell line (ab266119)

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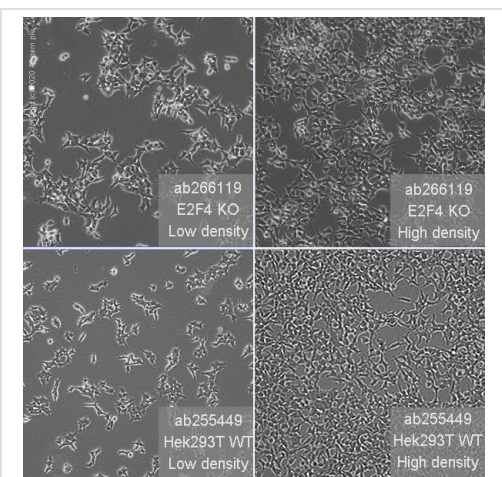
Mut  AAAAGTCCAAGAACAGCATC*****Insertion*****CAGT GGAAGT GAGT GGCAT
      |||
WT   AAAAGTCCAAGAACAGCATC          CAGT GGAAGT GAGT GGCAT

```

Allele-2: Insertion of the selection cassette in exon 2.

Sanger Sequencing - Human E2F4 knockout

HEK293T cell line (ab266119)



Human E2F4 knockout HEK293T cell line

(ab266119)

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