abcam

Product datasheet

# Human CRK (Crk p38) knockout HeLa cell line ab265097

2 Images

## Overview

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|---|---|
| **Product name** | Human CRK (Crk p38) knockout HeLa cell line |
| **Parental Cell Line** | HeLa |
| **Organism** | Human |
| **Mutation description** | Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 1 |
| **Passage number** | <20 |
| **Knockout validation** | Sanger Sequencing, Western Blot (WB) |
| **Tested applications** | **Suitable for:** WB |
| **Biosafety level** | 2 |
| **General notes** | **Recommended control:** Human wild-type HeLa cell line (**ab255928**). 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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We will provide viable cells that proliferate on revival.

## Properties

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|---|---|
| **Number of cells** | 1 x $10^6$ cells/vial, 1 mL |
| **Adherent /Suspension** | Adherent |
| **Tissue** | Cervix |
| **Cell type** | epithelial |
| **Disease** | Adenocarcinoma |
| **Gender** | Female |
| **STR Analysis** | Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8,12 CSF1PO: 9, 10 |
| **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** | The Crk-I and Crk-II forms differ in their biological activities. Crk-II has less transforming activity than Crk-I. Crk-II mediates attachment-induced MAPK8 activation, membrane ruffling and cell motility in a Rac-dependent manner. Involved in phagocytosis of apoptotic cells and cell motility via its interaction with DOCK1 and DOCK4. |
| **Sequence similarities** | Belongs to the CRK family.<br>Contains 1 SH2 domain.<br>Contains 2 SH3 domains. |
| **Domain** | The C-terminal SH3 domain function as a negative modulator for transformation and the N-terminal SH3 domain appears to function as a positive regulator for transformation.<br>The SH2 domain mediates interaction with SHB. |
| **Post-translational modifications** | Phosphorylation of Crk-II (40 kDa) gives rise to a 42 kDa form.<br>Phosphorylated on Tyr-221 upon cell adhesion. Results in the negative regulation of the association with SH2- and SH3-binding partners, possibly by the formation of an intramolecular interaction of phosphorylated Tyr-221 with the SH2 domain. This leads finally to the down-regulation of the Crk signaling pathway. |
| **Cellular localization** | Cytoplasm. Cell membrane. Translocated to the plasma membrane upon cell adhesion. |

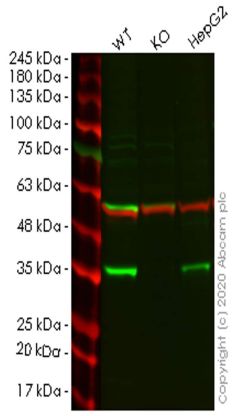
## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab265097 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: 33 kDa. |

## Images




Western blot - Human CRK knockout HeLa cell line (ab265097)

**All lanes :** Anti-Crk p38 antibody [EP242Y] (**ab45136**) at 1/1000 dilution

**Lane 1 :** Wild-type HeLa cell lysate
**Lane 2 :** CRK knockout HeLa cell lysate
**Lane 3 :** HepG2 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:** 33 kDa
**Observed band size:** 38 kDa

**Lanes 1-3:** Merged signal (red and green). Green - **ab45136** observed at 38 kDa. Red - loading control **ab7291** observed at 50 kDa.

**ab45136** Anti-Crk p38 antibody [EP242Y] was shown to specifically react with Crk in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265097 (knockout cell lysate **ab257899**) was used. Wild-type and Crk knockout samples were subjected to SDS-PAGE. **ab45136** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) were incubated overnight at 4°C 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.


Sanger Sequencing - Human CRK knockout HeLa cell line (ab265097)

Homozygous: 1 bp insertion in exon 1.

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

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