

# Human CASP3 (Caspase-3) knockout HeLa cell line ab255370

## 2 图像

### 概述

产品名称	人CASP3 (Caspase-3) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: Insertion of the selection cassette in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
经测试应用	适用于: WB
Biosafety level	2
常规说明	<p><b>Recommended control:</b> Human wild-type HeLa cell line (<a href="#">ab255448</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.

## 性能

Number of cells	1 x 10 <sup>6</sup> 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
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

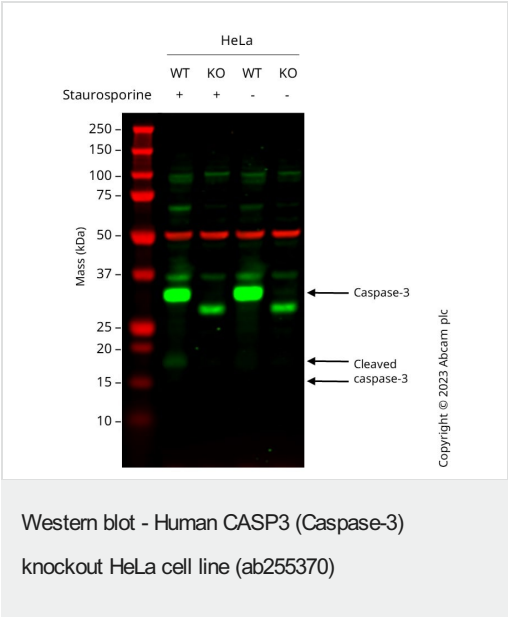
功能	Involved in the activation cascade of caspases responsible for apoptosis execution. At the onset of apoptosis it proteolytically cleaves poly(ADP-ribose) polymerase (PARP) at a '216-Asp-Gly-217' bond. Cleaves and activates sterol regulatory element binding proteins (SREBPs) between the basic helix-loop-helix leucine zipper domain and the membrane attachment domain. Cleaves and activates caspase-6, -7 and -9. Involved in the cleavage of huntingtin.
组织特异性	Highly expressed in lung, spleen, heart, liver and kidney. Moderate levels in brain and skeletal muscle, and low in testis. Also found in many cell lines, highest expression in cells of the immune system.
序列相似性	Belongs to the peptidase C14A family.
翻译后修饰	Cleavage by granzyme B, caspase-6, caspase-8 and caspase-10 generates the two active subunits. Additional processing of the propeptides is likely due to the autocatalytic activity of the activated protease. Active heterodimers between the small subunit of caspase-7 protease and the large subunit of caspase-3 also occur and vice versa. S-nitrosylated on its catalytic site cysteine in unstimulated human cell lines and denitrosylated upon activation of the Fas apoptotic pathway, associated with an increase in intracellular caspase activity. Fas therefore activates caspase-3 not only by inducing the cleavage of the caspase zymogen to its active subunits, but also by stimulating the denitrosylation of its active site thiol.
细胞定位	Cytoplasm.

## 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.

图片



**All lanes** : Anti-Caspase-3 antibody [EPR18297] ([ab184787](#)) at 1/2000 dilution

**Lane 1** : Wild-type HeLa Treated Staurosporine (2 uM, 4h) cell lysate

**Lane 2** : CASP3 knockout HeLa Treated Staurosporine (2 uM, 4h) cell lysate

**Lane 3** : Wild-type HeLa Vehicle Control Staurosporine (0 uM, 4h) cell lysate

**Lane 4** : CASP3 knockout HeLa Vehicle Control Staurosporine (0 uM, 4h) cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

**All lanes** : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

**Observed band size:** 35 kDa

Anti-CASP3 antibody [EPR18297] ([ab184787](#)) staining at 1/2000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] ([ab7291](#)) loading control staining at 1/20000 dilution, shown in red. In Western blot, [ab184787](#) was shown to bind specifically to CASP3. A band was observed at 35 kDa in treated wild-type HeLa cell lysates with a band observed at lower molecular weight in CASP3 knockout cell line ab255370 (knockout cell lysate [ab263779](#)) which cannot be cleaved to active CASP3. To generate this image, wild-type and CASP3 knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were

blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) 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 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

Mut	AATGGATTTTGAATCCACTG*****Insertion*****AGTTTTCAGTGTTCCTCATG
WT	AATGGATTTTGAATCCACTGAGTTTTCAGTGTTCCTCATG

Sanger Sequencing - Human CASP3 knockout HeLa cell line (ab255370)

Homozygous: Insertion of the selection cassette in exon 2.

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

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