

### Human FADD knockout HeLa cell lysate ab257261

画像数 3

#### 製品の概要

製品名	Human FADD knockout HeLa cell lysate
製品の概要	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: Insertion of the selection cassette in exon 1.
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. <i>*Usage of SDS sample buffer is not recommended with these lyophilized lysates.</i>

#### 特記事項

**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found [here](#). Please refer to our lysis protocol for further details on how our lysates are prepared.

**User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage.

Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. [See here for more information on knockout cell lysates.](#)

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#### アプリケーション

**適用あり:** WB

## 製品の特性

### 保存方法

Store at -80°C. Please refer to protocols.

内容	1 kit
ab260164 - Human FADD knockout HeLa cell lysate	1 x 100µg
ab255929 - Human wild-type HeLa cell lysate	1 x 100µg

### 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

## ターゲット情報

### 機能

Apoptotic adaptor molecule that recruits caspase-8 or caspase-10 to the activated Fas (CD95) or TNFR-1 receptors. The resulting aggregate called the death-inducing signaling complex (DISC) performs caspase-8 proteolytic activation. Active caspase-8 initiates the subsequent cascade of caspases mediating apoptosis.

### 組織特異性

Expressed in a wide variety of tissues, except for peripheral blood mononuclear leukocytes.

### 配列類似性

Contains 1 death domain.

Contains 1 DED (death effector) domain.

### ドメイン

Contains a death domain involved in the binding of the corresponding domain within Fas receptor.

## アプリケーション

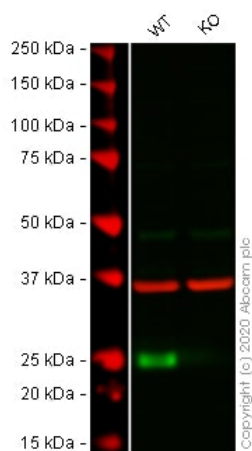
### The Abpromise guarantee

**Abpromise保証は、次のテスト済みアプリケーションにおけるab257261の使用に適用されます**

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 23 kDa.

## 画像



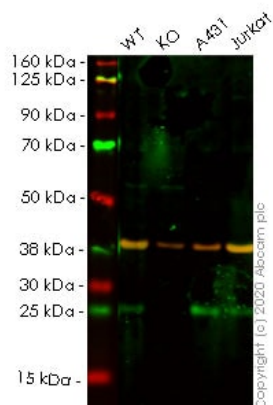
Western blot - Human FADD knockout HeLa cell lysate (ab257261)

**Lane 1:** Wild-type HeLa cell lysate 20 ug

**Lane 2:** FADD knockout HeLa cell lysate 20 ug

**Lanes 1 - 2:** Merged signal (red and green). Green - **ab108601** observed at 23 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

**ab108601** was shown to react with FADD in wild-type HeLa cells in western blot with loss of signal observed in FADD knockout cell line **ab261817** (FADD knockout cell lysate ab257261). Wild-type and FADD knockout HeLa cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab108601** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively.. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human FADD knockout HeLa cell lysate (ab257261)

**Lane 1:** Wild-type HeLa cell lysate (20 µg)

**Lane 2:** FADD knockout HeLa cell lysate (20 µg)

**Lane 3:** A431 cell lysate (20 µg)

**Lane 4:** Jurkat cell lysate (20 µg)

**Lanes 1-4:** Merged signal (red and green). Green - **ab119059** observed at 25 kDa. Red - loading control **ab181602** observed at 37 kDa.

**ab119059** Anti-FADD antibody [OT11C11] was shown to specifically react with FADD in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab261817** (knockout cell lysate ab257261) was used. Wild-type and FADD knockout samples were subjected to SDS-PAGE. **ab119059** and Anti-GAPDH antibody[EPR16891] - Loading Control (**ab181602**) were incubated overnight at 4°C at 1 in 2000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Mut	CCCGAGGCATAGGAATTGA****Insertion****GCTCGGTCAGCTCGCTGCTC
WT	CCCGAGGCATAGGAATTGA
	GCTCGGTCAGCTCGCTGCTC
Sanger Sequencing - Human FADD knockout HeLa cell lysate (ab257261)	

Homozygous: Insertion of the selection cassette in exon 1

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