

Human CDK6 knockout HeLa cell line ab266059

画像数 6

製品の概要

| | |
|-----------------------------|--|
| 製品名 | Human CDK6 knockout HeLa cell line |
| Parental Cell Line | HeLa |
| Organism | Human |
| Mutation description | Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 2 and Insertion of the selection cassette in exon 2 |
| Passage number | <20 |
| Knockout validation | Sanger Sequencing, Western Blot (WB) |
| アプリケーション | 適用あり: WB |
| Biosafety level | 2 |
| 特記事項 | <p>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.</p> <p>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: DMEM (High Glucose) + 10% FBS</p> <p>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.</p> <ol style="list-style-type: none"> 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 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. 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 2×10^4 cells/cm². Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO₂. Cultures should be monitored daily. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of 2×10^4 cells/cm² 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.

製品の特性

| | |
|----------------------|---|
| 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 |
| 保存方法 | Shipped on Dry Ice. Store in liquid nitrogen. |
| バッファー | Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether |

ターゲット情報

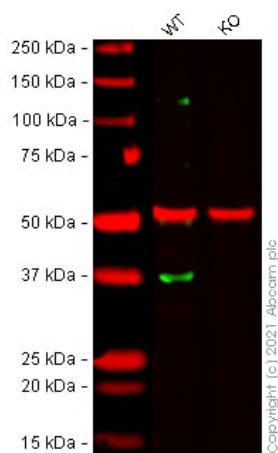
| | |
|-------|--|
| 機能 | Probably involved in the control of the cell cycle. Interacts with D-type G1 cyclins. |
| 配列類似性 | Belongs to the protein kinase superfamily. CMGC Ser/Thr protein kinase family. CDC2/CDKX subfamily. Contains 1 protein kinase domain. |

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ab266059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: 37 kDa. |

画像



Western blot - Human CDK6 knockout HeLa cell line (ab266059)

All lanes : Anti-Cdk6 antibody [98D] (**ab241554**) at 1/2000 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2 : CDK6 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

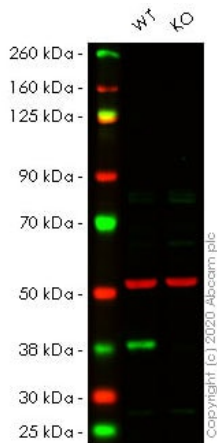
Performed under reducing conditions.

Predicted band size: 37 kDa

Observed band size: 38 kDa

Lanes 1 - 2: Merged signal (red and green). Green - **ab241554** observed at 38 kDa. Red - loading control **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55 kDa.

ab241554 was shown to react with Cdk6 in HeLa wild-type cells in Western blot with loss of signal observed in CDK6 knockout cell line ab266059 (CDK6 knockout cell lysate **ab257088**). Wild-type HeLa and CDK6 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab241554** and **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4°C at a 1 in 2000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Human CDK6 knockout HeLa cell line (ab266059)

All lanes : Anti-Cdk6 antibody [8H4] ([ab54576](#))

Lane 1 : Wild-type HeLa cell lysate

Lane 2 : CDK6 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

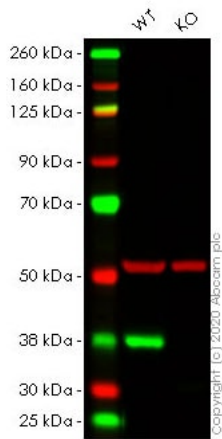
Performed under reducing conditions.

Predicted band size: 37 kDa

Observed band size: 37 kDa

Lanes 1- 2: Merged signal (red and green). Green - [ab54576](#) observed at 37 kDa. Red - Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker ([ab52866](#)) observed at 50 kDa.

[ab54576](#) was shown to react with Cdk6 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab266059 (knockout cell lysate [ab257088](#)) was used. Wild-type HeLa and Cdk6 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. [ab54576](#) and Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker ([ab52866](#)) overnight at 4°C at 1 µg/ml and a 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.



Western blot - Human CDK6 knockout HeLa cell line (ab266059)

All lanes : Anti-Cdk6 antibody [EPR4515] (**ab124821**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2 : CDK6 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 37 kDa

Observed band size: 37 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab124821** observed at 37 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

ab124821 was shown to react with Cdk6 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab266059 (knockout cell lysate **ab257088**) was used. Wild-type HeLa and Cdk6 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab124821** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) overnight at 4°C at a 1 in 1000 dilution and a 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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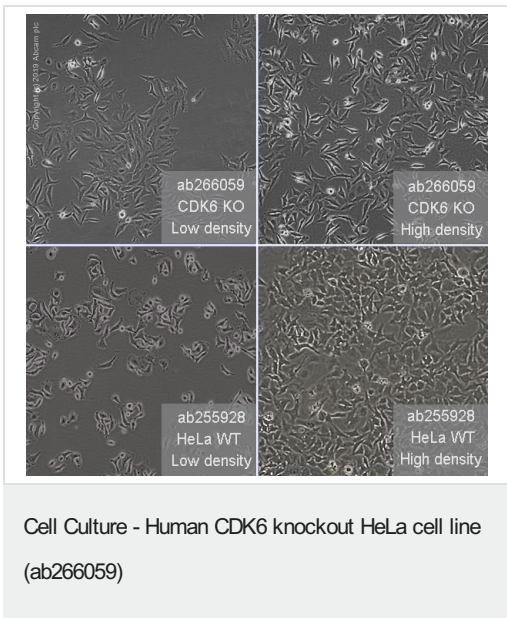
Mut  GGCATGGAGAAGGACGGCCTGTGCCGCGCTTGACCAGCAGTACGAATGCGTGGC GGAGAT
      |||
WT   GGCATGGAGAAGGACGGCCTGTGCCGCGCTTGACCAGCAGTACGAATGCGTGGC GGAGAT
  
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Sanger Sequencing - Human CDK6 knockout HeLa cell line (ab266059)

Allele-1: 1 bp insertion in exon2



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



Representative images of CDK6 knockout HeLa cells, low and high confluency examples (top left and right respectively) and wild-type HeLa 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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