# abcam

## **Product datasheet**

# Human MFN2 (Mitofusin 2) knockout HEK-293 cell line ab260861

### 画像数 2

#### 製品の概要

| 製品名                  | Human MFN2 (Mitofusin 2) knockout HEK-293 cell line                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |  |  |
|----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|--|
| Parental Cell Line   | HEK-293                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |  |  |
| Organism             | Human                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |  |
| Mutation description | Knockout achieved by CRISPR/Cas9; X = 10 bp deletion                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |  |  |
| Passage number       | <20                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |  |  |
| Knockout validation  | Next Generation Sequencing (NGS), Western Blot (WB)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |  |  |
| アプリケーション             | 適用あり: Next Generation Sequencing, WB                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |  |  |
| Biosafety level      | 2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |  |  |
| 特記事項                 | <b>Recommended control:</b> Human wild-type HEK-293 cell line ( <u>ab259776</u> ). Please note a wild-<br>type cell line is not automatically included with a knockout cell line order, if required please add<br>recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |  |  |
|                      | <b>Cryopreservation cell medium:</b> Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |  |  |
|                      | Culture medium: DMEM (High Glucose) + 10% FBS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |  |  |
|                      | <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.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |  |  |
|                      | <ol> <li>Thaw the vial in 37°C water bath for approximately 1-2 minutes.</li> <li>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>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<sup>4</sup> 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>Incubate the culture at 37°C incubator with 5% CO<sub>2</sub>. Cultures should be monitored daily.</li> </ol> |  |  |
|                      | <b>Subculture guidelines:</b><br>All seeding densities should be based on cell counts gained by established methods.<br>A guide seeding density of 2x10 <sup>4</sup> cells/cm <sup>2</sup> 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. This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.

We will provide viable cells that proliferate on revival.

#### 製品の特性

| Number of cells      | 1 x 10 <sup>6</sup> cells/vial, 1 mL                             |  |
|----------------------|------------------------------------------------------------------|--|
| Adherent /Suspension | Adherent                                                         |  |
| Tissue               | Kidney                                                           |  |
| Cell type            | epithelial                                                       |  |
| Gender               | Female                                                           |  |
| 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

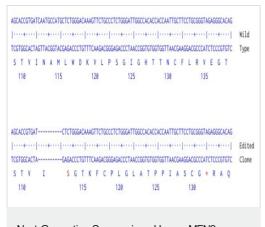
| 機能    | Essential transmembrane GTPase, which mediates mitochondrial fusion. Fusion of mitochondria occurs in many cell types and constitutes an important step in mitochondria morphology, which is balanced between fusion and fission. MFN2 acts independently of the cytoskeleton. It therefore plays a central role in mitochondrial metabolism and may be associated with obesity and/or apoptosis processes. Overexpression induces the formation of mitochondrial networks. Plays an important role in the regulation of vascular smooth muscle cell proliferation. Involved in the clearance of damaged mitochondria via selective autophagy (mitophagy). Is required for PARK2 recruitment to dysfunctional mitochondria. Involved in the control of unfolded protein response (UPR) upon ER stress including activation of apoptosis and autophagy during ER stress. Acts as an upstream regulator of EIF2AK3 and suppresses EIF2AK3 activation under basal conditions. |  |
|-------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
| 組織特異性 | Ubiquitous; expressed at low level. Highly expressed in heart and kidney.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |  |
| 関連疾患  | Charcot-Marie-Tooth disease 2A2<br>Neuropathy, hereditary motor and sensory, 6A                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |  |
| 配列類似性 | Belongs to the TRAFAC class dynamin-like GTPase superfamily. Dynamin/Fzo/YdjA family.<br>Mitofusin subfamily.<br>Contains 1 dynamin-type G (guanine nucleotide-binding) domain.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |  |
| 翻訳後修飾 | Phosphorylated by PINK1.<br>Ubiquitinated by non-degradative ubiquitin by PARK2, promoting mitochondrial fusion;<br>deubiquitination by USP30 inhibits mitochondrial fusion.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |  |
| 細胞内局在 | Mitochondrion outer membrane. Colocalizes with BAX during apoptosis.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |  |

#### Abpromise保証は、次のテスト済みアプリケーションにおけるab260861の使用に適用されます

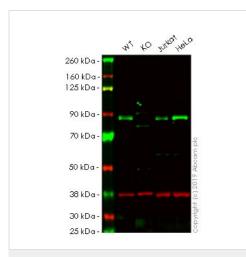
#### アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

| アプリケーション                      | Abreviews | 特記事項                                     |
|-------------------------------|-----------|------------------------------------------|
| Next Generation<br>Sequencing |           | Use at an assay dependent concentration. |
| WB                            |           | Use at an assay dependent concentration. |

画像



Next Generation Sequencing - Human MFN2 (Mitofusin 2) knockout HEK-293 cell line (ab260861)



Western blot - Human MFN2 (Mitofusin 2) knockout HEK-293 cell line (ab260861)

#### 10 bp deletion after lle112 of the WT protein

All lanes : Anti-Mitofusin 2 antibody [EPR19796] (<u>ab205236</u>) at 1/2000 dilution

Lane 1 : Wild-type HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 2 : MFN2 knockout HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 3 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate
Lane 4 : HeLa (Human epithelial cell line from cervix

adenocarcinoma) whole cell lysate

Lysates/proteins at 20 µg per lane.

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab205236</u> observed at 86 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

ab205236 was shown to recognize MFN2 (Mitofusin 2) in wild-type

HEK-293 cells as signal was lost at the expected MW in MFN2 knockout cell line ab260861 (knockout cell lysate **ab261653**). Additional cross-reactive bands were observed in the wild-type and knockout samples. Wild-type and MFN2 knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% milk. Ab205236 and **ab8245** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/2000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye<sup>®</sup> 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

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