



## HEK293 - TMEM163 (KO) Cell Line

Cat. No.: NTV-0925-YJ750

This product is for research use only and is not intended for diagnostic use.

### Summary

<b>Description</b>	This HEK293 cell line has been precisely engineered to ablate the expression of the TMEM163 gene. This cell line was generated using the CRISPR/Cas9 system. It serves as an ideal model for investigating the functional roles of TMEM163.
<b>Species</b>	Human
<b>Size</b>	1×10 <sup>6</sup> cells
<b>Growth Conditions</b>	37°C with 5% CO <sub>2</sub>
<b>Related Disease</b>	Parkinson's disease
<b>Synonyms</b>	TMEM163 knockout HEK293 cells; TMEM163-deficient 293 line; HEK293 TMEM163-null cells; TMEM163 KO in HEK293 cells; 293 cells lacking TMEM163
<b>Parental cells</b>	HEK293
<b>Format</b>	Frozen
<b>Shipping</b>	Dry Ice
<b>Storage</b>	Store in liquid nitrogen.

### Specifications

<b>Mycoplasma Testing</b>	Negative
<b>Recovery of Frozen Cells</b>	<ol style="list-style-type: none"><li>1. Preparation: Preheat a 37°C water bath and the complete culture medium. Pipette 6 mL of the pre-warmed medium into a 15 mL conical tube and keep it ready.</li><li>2. Thawing:</li></ol>

For safety, first move the cryovial from liquid nitrogen to a -80°C freezer for 10 minutes. Then, rapidly thaw the vial in the 37°C water bath by gently swirling it for under 1 minute until only a small ice crystal remains. Ensure the cap does not contact the water.

### 3. Aseptic Transfer and Dilution:

Inside a biosafety cabinet, sterilize the outside of the vial with 75% alcohol. Transfer the entire thawed cell suspension into the prepared conical tube with medium. To maximize recovery, rinse the empty vial with 1 mL of medium and add it to the same tube.

### 4. Centrifugation:

Pellet the cells by centrifuging the suspension, typically at 1100 rpm for 4 minutes. (Note: Optimal speed and time are cell-type dependent).

### 5. Resuspension and Plating:

Carefully aspirate and discard the supernatant containing the cryoprotectant. Gently resuspend the cell pellet in 1 mL of fresh complete medium. Transfer the cells to a T25 culture flask and add fresh medium to a total volume of at least 6 mL.

### 6. Incubation:

Gently mix the cells in the flask to ensure even distribution and place it in a humidified incubator set to 37°C and 5% CO<sub>2</sub>.

### 7. Next-Day Care for Adherent Cells:

The following day, check for cell attachment. If attached, replace the old medium with fresh complete medium. If cells are floating but appear viable (round and bright), wait another 24 hours before changing the medium. Afterwards, refresh the medium every 2-3 days and passage when confluence is over 80%.

### 8. Next-Day Care for Suspension Cells:

The following day, assess cell health. If viable, refresh the medium. For poor recovery, consider using a smaller flask and a higher serum concentration (e.g., 20%). If cells still appear unhealthy after 72 hours, contact technical support for assistance.

---

## Subculturing Procedure

### 1. Preparation

Preheat the complete culture medium, PBS, and trypsin-EDTA solution to 37°C.

### 2. Wash Cells

Aspirate and discard the old culture medium from the flask. Gently rinse the cell monolayer once with pre-warmed PBS (e.g., ~6 mL for a T25 flask), then discard the PBS.

### 3. Cell Detachment

Add enough trypsin-EDTA to cover the cell layer (e.g., ~3 mL for a T25 flask) and incubate at 37°C. Monitor under a microscope until about 70-80% of the cells become rounded, then gently tap the flask to dislodge them.

### 4. Neutralize Trypsin

Quickly add at least a double volume of pre-warmed complete medium (e.g., 6 mL for a T25 flask) to the flask to inactivate the trypsin. Gently pipette the medium over the surface to ensure all cells are dislodged and suspended.

### 5. Collect and Pellet Cells

Transfer the entire cell suspension to a sterile conical tube. To maximize yield, you can rinse the flask with a small amount of PBS and add it to the tube. Centrifuge the suspension (e.g., 1100 rpm for 4 minutes) to form a cell pellet.

#### 6. Resuspend and Seed

Carefully discard the supernatant. Gently resuspend the cell pellet in a small volume of fresh complete medium. Add the resuspended cells to new culture flasks at your desired split ratio (a 1:3 ratio is recommended for the first passage).

#### 7. Incubation

Add the appropriate volume of fresh medium to the new flasks. Place them in a humidified incubator at 37°C with 5% CO<sub>2</sub>. Ensure proper gas exchange by either loosening the flask caps or using vented caps.

#### 8. Post-Passage Maintenance

Check the cells the following day. If there is a significant number of floating dead cells, replace the medium. Continue to monitor cell growth and change the medium as needed, passaging again once the cells reach over 80% confluence.

---

**Cryopreservation Medium** 95% Complete Culture Medium with 5% DMSO

---

**Research Use Only** For Research Use Only. Not For Clinical Use.

---