

Description

The TetR HEK293 Cell Line is a HEK293 cell line expressing the repressor of the tetracycline resistance element (TetR), creating an inducible system based on the presence of Tetracycline or Doxycycline in the cell culture media. These cells can be transfected with a target of interest under the control of a strong promoter and the Tetracycline Operon. In the absence of tetracycline or doxycycline, TetR binds to the operon and represses transcription. When exposed to tetracycline or doxycycline, TetR loses affinity for the tetracycline resistance element expression of the target of interest occurs.

This cell line has been validated by stimulation with doxycycline.

Background

TetR, or Tet repressor proteins play a role in development of antibiotic resistance to tetracycline (Tc). Tc are a family of antibiotics that kills bacteria by interfering with protein synthesis. Resistance to Tc occurs by expression of Tc resistance genes, which are regulated by TetR. In addition to the study of mechanisms of antibiotic resistance, the use of TetR has been used as a mode to regulate promoter expression, and thus create inducible cell models. These inducible cell models have been crucial for the understanding of cellular pathways.

Application

Transient tetracycline-inducible protein expression of a target of interest.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains $>1 \times 10^6$ cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent.

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1H	BPS Bioscience #79546

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.



Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest.

Cells should be grown at 37°C with 5% CO₂. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1H (BPS Bioscience #79546):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, plus 5 µg/ml of Blasticidin.

Cell Culture Protocol

Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 1.

Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1.
3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO₂ incubator.
4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 and continue growing in a 5% CO₂ incubator at 37°C until the cells are ready to passage.
5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1H.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca²⁺/Mg²⁺, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 1H and transfer to a tube.
3. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1H.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:8 to 1:20 weekly or twice per week.

Note: Just after thawing and when cells are at low density, the cells may grow at a slower rate. It is recommended to split the cells at a 1:4 ratio in those cases. After several passages, the cell growth rate increases, and the cells can be split using a higher ratio.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without $\text{Ca}^{2+}/\text{Mg}^{2+}$ and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 1H and count the cells.
3. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at $\sim 2 \times 10^6$ cells/ml.
4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.
5. Transfer the vials to liquid nitrogen the next day for long term storage.



Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

References

Dobrovolsky V.N. and Heflich R.H., 2007 *Biotechnol Bioeng.* 98(3):719-23.

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product.

Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	Catalog #	Size
TRPM-2 HEK293 Cell Line	90331	2 vials
CSL Reporter HEK293 Cell Line	79754	2 vials
RFP/GFP Safe Harbor HEK293 Cell Line	78581	2 vials
Cas9/GFP Safe Harbor HEK293 Cell Line	78582	2 vials
GAL4 Luciferase Reporter HEK293 Cell Line	60656	2 vials
GR-GAL4 Luciferase Reporter HEK293 Cell Line	60655	2 vials

Version 110723