A549-RepTor™ Cells

Tetracycline repressor (TetR) expressing A549 cells

Catalog code: a549-rtor

https://www.invivogen.com/tet-on-a549-reptor-cells

For research use only

Version 24B14-NJ

PRODUCT INFORMATION

Contents

- **3-7 x 10^6 of A549-RepTor™ cells in a cryovial or shipping flask.** *IMPORTANT: If cells provided in a cryovial are not frozen upon arrival, contact InvivoGen immediately.*
- **1 mg of Doxycycline**. Store at -20 °C.
- **1 ml of Blasticidin** (10 mg/ml). Store at 4 °C or at -20 °C.*
- **1 ml of Normocin™** (50 mg/ml), a formulation of three antibiotics active against mycoplasmas, bacteria and fungi. Store at -20 °C.*

*The expiry date is specified on the product label.

Note: Data sheets for Blasticidin and Normocin™ are available on our website.

Handling Frozen Cells Upon Arrival

Cells must be thawed immediately upon receipt and grown according to handling procedures (as described on the next page) to ensure the best cell viability and proper assay performance.

*Note: **Avoid freezing cells upon receipt** as it may result in irreversible damage to the cell line.*

Disclaimer: We cannot guarantee cell viability if the cells are not thawed immediately upon receipt and grown according to handling procedures.

IMPORTANT: For cells that arrive in a shipping flask please refer to the enclosed 'cell recovery procedure'.

Cell Line Stability

Cells will undergo genotypic changes over time that will result in reduced responsiveness in normal cell culture conditions. Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages. A549-RepTor™ cells should not be passaged more than 20 times to remain fully efficient.

Quality Control

- Inducible reporter activity in the presence of doxycycline has been validated using A549-RepTor™ cells transfected with pTiGer2-SEAP.
- The stability for 20 passages following thawing has been verified.
- These cells are guaranteed mycoplasma-free.

USE RESTRICTIONS

These cells are distributed for research purposes only.

This product is covered by a Limited Use License. By use of this product, the buyer agrees the terms and conditions of all applicable Limited Use Label Licenses. For non-research use, such as screening, quality control or clinical development, contact outlicensing@invivogen.com.

1. **Kallunki, T. *et al.*, (2019).** How to choose the right inducible gene expression system for mammalian studies? Cells. DOI: 10.3390/cells8080796. **2. Hillen, W., Wissmann, A. (1989).** Tet repressor-tet operator interaction. Protein-Nucleic Acid Interaction. DOI: 10.1007/978-1-349-09871-2_7.

PRODUCT DESCRIPTION

A549-RepTor™ cells were engineered from the A549 human lung carcinoma cell line and designed for the inducible expression of a gene of interest (GOI). They feature strong and stable expression of the tetracycline repressor (TetR) in the nucleus. A549-RepTor™ cells are ready to be transfected with a GOI under the control of strong promoter and tetracycline operator (tetO) sequences. This can be achieved using InvivoGen's pTiGer-mcs plasmids. In these transfected cells, TetR binds to the tetO sequences and represses the gene transcription[1,2]. Upon incubation with doxycycline (a synthetic tetracycline derivative), TetR is released from the tetO sequences and the GOI is transcribed[1,2]. RepTor™ cells guarantee maximal repression of the GOI, ensuring minimal leakage of GOI expression in the absence of tetracycline/doxycycline, and strong GOI expression in the presence of the antibiotic.

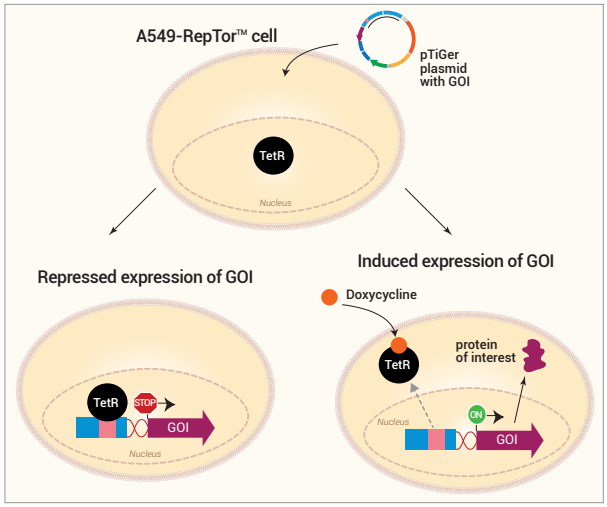
A549-RepTor™ cells are resistant to Blasticidin.

APPLICATIONS

The conditional expression of proteins in mammalian cells is favored over stable expression in a variety of basic and applied research areas, including functional genomics, gene therapy, biopharmaceutical protein production, and drug screening.

InvivoGen's inducible protein expression system is useful for, but not restricted to the following applications:

- Fine-tuning expression and functional study of individual genes
- Controlled expression of lethality-causing genes or gain-of-function variants
- Controlled production of toxic proteins
- Screening of specific inhibitors



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FAQ Any questions about our cell lines? Visit our FAQ page.

SAFETY CONSIDERATIONS

Biosafety Level 1

HANDLING PROCEDURES

Required Cell Culture Medium

- **Growth Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% (v/v) heat-inactivated fetal bovine serum (FBS, 30 min at 56 °C), 100 U/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml Normocin™
- **Test Medium:** DMEM, 4.5 g/l glucose, 2 mM L-glutamine, 10% (v/v) heat-inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin **without Normocin™ and Blasticidin**
- **Freezing Medium:** DMEM with 20% FBS and 10% (v/v) DMSO

Required Selective Antibiotic(s)

- Blasticidin

Initial Culture Procedure

The first propagation of cells should be for generating stocks for future use. This ensures the stability and performance of the cells for subsequent experiments.

1. Thaw the vial by gentle agitation in a 37 °C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid.
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% (v/v) ethanol. *Note: All steps from this point should be carried out under strict aseptic conditions.*
3. Transfer cells in a tube containing 15 ml of pre-warmed growth medium. **Do not add selection antibiotics until the cells have been passaged twice.**
4. Centrifuge the vial at 150 x g (RCF) for 10 minutes.
5. Remove the supernatant containing cryoprotective agent and resuspend cells with 1 ml of growth medium without selection antibiotics.
6. Transfer the vial contents to a 25 cm^2 tissue culture flask containing 5 ml of growth medium without selection antibiotics.
7. Place the culture at 37 °C in 5% CO_2.

Frozen Stock Preparation

1. Resuspend cells at a density of 5-7 x 10^6 cells/ml in freezing medium freshly prepared with cold growth medium.

Note: A T-75 culture flask typically yields enough cells for preparing 3-4 frozen vials.

2. Prepare 1 ml aliquots of cells in cryogenic vials.
3. Place vials in a freezing container and store at -80 °C overnight.
4. Transfer vials to liquid nitrogen for long term storage.

Note: If properly stored, cells should remain stable for years.

Cell Maintenance

1. A549-RepTor™ cells grow as adherent cells. Detach the cells using trypsin for 2-3 min at room temperature (RT).

Note: Prolonged action of trypsin or incubation at 37°C may alter the cell surface expression of receptors.

2. After the cells have recovered (after at least one passage), subculture the cells in growth medium supplemented with 10 µg/ml Blasticidin.
3. Renew growth medium twice a week.
4. Cells should be passaged when a 70-80% confluency is reached. Do not let the cells grow to 100% confluency.

Note: To ensure the best results, use A549-RepTor™ cells with less than 20 passages.

INDUCTION OF GENE OF INTEREST

Below is a protocol to work with A549-Reptor™ cells transfected with a tetracycline-inducible gene of interest (GOI) cloned into a pTiGer-mcs plasmid.

Plating cells (example for a 24-well plate)

1. Detach A549-RepTor™ cells using trypsin for 2-3 min at RT. Resuspend cells in fresh, pre-warmed test medium and prepare a cell suspension at ~200,000 cells/ml.

Note: The response of A549-RepTor™ cells can be altered by the prolonged action of trypsin. Do not incubate with trypsin at 37°C and for no longer than 2-3 min.

2. Add ~200,000 cells (1 ml) per well of a 24-well plate.
3. Incubate the plate overnight at 37 °C in a 5% CO_2 incubator.

Transfection

1. Prepare a transfection complex using your pTiGer construct and a cationic lipid reagent (e.g. Lipofectamine® LTX) according to the manufacturer's instructions.

Note: The user should assess different ratios of transfection complex for optimal epxression without leakage. We recommend to use lower ratios (e.g. 0.3 µg/100 µl) for transient transfection and higher ratios (e.g. 1 µg/100 µl) for stable transfection

Note: The user may add NATE™ 1X, a nucleic acid transfection enhancer specifically designed to increase transfection efficiency.

2. Carefully remove all medium from wells and replace with freshly prepared and pre-warmed test medium. Proceed to the transfection according to the manufacturer's instructions.
3. Incubate the plate 24 hours at 37 °C in a 5% CO_2 incubator.
4. After 24 hours, you may proceed to the induction of gene expression with the transiently transfected cells.

Alternatively, you may generate a stable cell line using selective antibiotics when passaging the cells.

Note: Prior to establishing a stable cell line with your GOI, your pTiGer construct should be tested for functionality. Transiently transfect the A549-RepTor™ cells and test for GOI induction with doxycycline (Dox).

Induction of gene expression

1. Prepare Dox solution using pre-warmed test medium.

Note: The user should determine the minimal concentration of Dox that is required for high GOI expression. You may view examples in the cell line validation data sheet.

2. Carefully remove all medium from wells and add Dox.
3. Incubate the plate at 37 °C in a 5% CO_2 incubator.

Note: The user should assess the optimal incubation duration with Dox depending on the GOI.

4. Assess the GOI expression (e.g. Western Blot or flow cytometry).

RELATED PRODUCTS

Product	Description	Cat.Code
Blasticidin	Selection antibiotic	ant-bl-05
pTiGer2-mcs	Muligenic coding plasmid	ptg2-mcs
NATE™	Nucleic acid transfection enhancer	lyec-nate
HEK-RepTor™ cells	TetR-expressing cell line	hk-rtor