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Product Information

CompoZr® ADME/Tox Cell Lines
Human RPTEC Control Cells (SA7K Clone)

Catalog Number **MTOX1030**
Store Temperature –130 °C or below in liquid nitrogen vapor phase

TECHNICAL BULLETIN

Product Description

CompoZr® zinc finger nuclease (ZFN) technology is a fast and reliable way to manipulate the genome in a targeted fashion. ZFNs are naturally occurring proteins that can be engineered to bind DNA at a sequence-specific location and create a double strand break (www.sigma.com/zfn). The cell's natural machinery repairs the break in one of two ways: non-homologous end joining or homologous recombination. The non-homologous end joining pathway typically produces small modifications (indels) at the targeted locus that may result in a functional knockout. Single cell clones are then isolated, tested for the desired modification, and expanded to establish stable cell lines.

Kidney toxicity is a major concern during drug development. Although primary human kidney cells are available and a handful of immortalized kidney cell lines currently exist, there are concerns with passage limitations and limited functionality in these systems. Human primary renal proximal tubule epithelial cells (RPTEC) were modified with ZFNs to effectively extend cell proliferation. The resulting cell line was characterized for the presence of proximal tubule cell markers as well as several functional properties, including response to several known human nephrotoxicants similar to human primary cells and robust expression and activity of key renal uptake and efflux transporters incuding OCT2, OATP4C1, OAT1, OCTN1, MRP2, MRP4, P-gp, MATE1, MATE2-K, PEPT1, and PEPT2.

This product consists of ZFN engineered RPTEC SA7K Clone Control Cells. They are intended for use as both Control Cells for RPTEC Knockout Cells as well as for a wide variety of kidney cell based assays.

Component

Human RPTEC Control Cells (SA7K Clone) 1 vial
Catalog Number MTOX1030
Vial contains $\geq 3 \times 10^6$ modified RPTEC cells.

Note: **Neither media nor supplements are supplied** with the vials. These must be obtained prior to receiving the vials.

The cryoprotectant medium used is Cell Freezing Medium-DMSO 1×, Catalog No. C6164

Parental Cell Line: Zen Bio HK016
Note: Please see product datasheet from Zen Bio for additional information about the origin of these cell lines. Cytogenetic information is based on initial seed stock at Sigma Life Science. Cytogenetic instability has been reported in the literature for some cell lines.

Cell Line Description
Clone: RPTC-HK016 Zen Bio
Organism*: Homo sapiens* (human)

Tissue: Renal Proximal Convoluted Tubule Epithelial Cells

Age: 8.8 years

Gender: Female

Ethnicity: African American

Morphology: Epithelial

Growth properties: Adherent

Species-specific PCR Evaluation:
The cells were confirmed to be of human origin and no mammalian interspecies contamination was detected.

PCR Evaluation for *Mycoplasma* species contamination: Negative

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
Upon receiving a shipment of frozen cells it is important the end user gives the shipment attention without delay. To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70 °C. Storage at –70 °C will result in loss of viability.

Precaution: It is recommended that protective gloves and clothing always be used, and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to the gas phase may result in the rapid expansion of the vessel, potentially blowing off its cap with dangerous force creating flying debris. At the time a cell line is ordered, end users should also consider the culture conditions for the new cell line and make sure the appropriate medium will be available when the cells arrive.

Procedures
Protocol for Thawing and Seeding 24 Well Plates
Note: One cryovial of Human RPTEC Control Cells (SA7K Clone) contains enough cells to seed one plate.

Reagents and Equipment Required but Not Provided for Thawing and Seeding
Note: Neither media nor supplements are supplied with the vials. These must be obtained prior to receiving the vials.

- RPTEC Complete Supplement (Catalog Number MTOXRCSUP – 30 mL)
- RPTEC Tox Supplement (Catalog Number MTOXRTSUP – 6 mL)
- Minimum Essential Medium Eagle Alpha Modification Medium (MEMα) (Catalog Number M4526)
- L-glutamine (Catalog Number G7513)
- Gentamicin solution (Catalog Number G1397)
- Amphotericin B solution (Catalog Number A2942)
- BSL-2 hood
- Cell culture incubator

Medium Preparation
Prepare Complete Medium by supplementing 500 mL of MEMα (Catalog Number M4526) with 6.25 mL of L-glutamine (Catalog Number G7513), 30 mL of RPTEC Complete Supplement (Catalog Number MTOXRCSUP), 0.3 mL of Gentamicin solution (Catalog Number G1397), and 0.03 mL of Amphotericin B solution (Catalog Number A2942). Complete Medium can be stored at 2–8 °C for up to 1 month. This medium is formulated for use with a 5% CO_2 in air atmosphere.

Prepare Tox Medium by supplementing 500 mL of MEMα (Catalog Number M4526) with 6.25 mL of L-glutamine (Catalog Number G7513), 6 mL of RPTEC Tox Supplement (Catalog Number MTOXRTSUP), 0.3 mL of Gentamicin solution (Catalog Number G1397), and 0.03 mL of Amphotericin B solution (Catalog Number A2942). Tox Medium can be stored at 2–8 °C for up to 1 month. This medium is formulated for use with a 5% CO_2 in air atmosphere.

Thawing of Frozen Cells
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 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL of RPTEC Complete Medium and spin at 125 × *g* for 3–5 minutes.
4. Resuspend cell pellet with the RPTEC Complete Medium. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested, prior to the addition of the vial contents, the culture vessel containing the REPTC Complete Medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0–7.6) and temperature (37 °C).

Transporter Assays

1. Resuspend cell pellet in 12 mL of RPTEC Complete Medium and add 0.5 mL per well of 24 well plate. Cells should be greater than 95% confluent after 2 days.

2. Uptake/Efflux Transporter Assays
   a. For assays involving the use of chemical inhibitors, add the desired concentration of the inhibitor to selected wells and pre-incubate for 30 minutes.
   b. Following the pre-incubation with inhibitors, add desired concentration of substrates and incubate for 30 minutes.
   c. Stop the reaction by washing the cells three times with ice-cold HBSS buffer.
   d. Prepare and analyze the samples according to established protocols.

Toxicity Assay

1. Resuspend cell pellet in 12 mL of RPTEC Complete Medium and add 0.5 mL per well of 24 well plate. Cells should be greater than 95% confluent after 2 days.
2. On day of treatment, change medium to Tox Medium. Prepare and dilute compound to generate a dose concentration response curve. Treat cells with appropriate volume of compound to obtain final concentrations.
3. After 48–72 hours, determine toxicity of compound. Note: The use of MTT Based *In Vitro* Toxicology Assay Kit (Catalog Number TOX1) is recommended.

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These products are covered by the "Consumable Vial" License as described in Exhibit 1.

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7. Products covered by a "Consumable Vial" License can only be incorporated in an end-use assay, seeded directly on the final assay plate (including, but not limited to 6-well, 12-well, 24-well, 48-well, or 96-well plates). All Products that are not utilized in an end-use assay plates shall be immediately destroyed.

8. Products delivered in a Petaka cell culture device can only be incorporated in an end-use assay for up to two (2) transwell plates (including, but not limited to 24-well or 96-well plates). All Products that are not utilized in an end-use assay for up to two (2) transwell plates shall be immediately destroyed.

9. Your right to use the Product will terminate immediately upon expiration of this License Agreement, or in the event that you fail to comply with these terms and conditions. You shall, upon such termination of your rights, destroy all Product, any modified versions of the Product, and components thereof in your control, and notify Sigma of such in writing.

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EXHIBIT 2

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commercial products or cDNA libraries or cell stock from the cells; will not use them for any study of a duration exceeding 20 days; and you will not license, resell or transfer them to anyone outside your organization for any reason. If you are unwilling to accept the terms of this LIMITED USE LICENSE, do not use the cells and return them to SA for credit. Violators of this LIMITED USE LICENSE will be prosecuted to the fullest extent of the law.