Altogen Biosystems

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Beta-TC6 Transfection Protocol (Kit for Pancreas Tumor Cells, CRL-11506)

Catalog No. 6150 Size: 0.5 ml Catalog No. 6151 Size: 1.5 ml Catalog No. 6152 Size: 8.0 ml

Contents and Shipping:

Beta-TC6 Transfection Kit includes Beta-TC6 Transfection Reagent (0.5 ml / 1.5 ml / 8.0 ml), Transfection Enhancer (0.5 ml), and Complex Condenser (0.5 ml). Beta-TC6 Transfection Reagent is supplied in liquid form at a concentration of 0.3 mg/ml, shipped at ambient temperature.

Description:

Two component lipid-based proprietary formulation optimized for Beta-TC6 cells transfection.

Product Qualification:

Beta-TC6 Transfection Reagent is tested functionally by transfection of Beta-TC6 cells with a small interfering RNAs targeting 3 different genes (Lamin A/C, GAPDH, Cyclophilin B). Transfection Reagent is tested for absence of nuclease contamination and microbial contamination.

Storage:

Store reagent at 4°C upon receipt. If stored properly, reagent is stable for 12 months.

Intended Use:

For *in vitro* use only.

Safety Data Sheet (SDS):

SDS documents are available online at https://altogen.com

In Vivo Transfection Kits (for compound testing in rodents):

- Catalog #5010 / 5011 / 5012 Lipid In Vivo Transfection Kit
- Catalog #5020 / 5021 / 5022 Polymer In Vivo Transfection Kit
- Catalog #5030 / 5031 / 5032 Nanoparticle In Vivo Transfection Kit
- Catalog #5040 / 5041 / 5042 PEG-Liposome In Vivo Transfection Kit
- Catalog #5050 / 5051 / 5052 Pancreas In Vivo Transfection Kit
- Catalog #5060 / 5061 / 5062 Liver In Vivo Transfection Kit
- Catalog #5070 / 5071 / 5072 Kidney In Vivo Transfection Kit

Transfection Controls and Recommended Products:

- Catalog #4060 GFP-expressing plasmid DNA (25 ug)
- Catalog #4061 Cell Cycle Arrest siRNA (5 nmol)
- Catalog #4062 Apoptosis Inducing siRNA (5 nmol)

To Place an Order:

Both domestic (USA) and international orders can be placed online (https://altogen.com) using credit card payment. Purchase Order (PO) can be faxed at (702) 989-0841 (for USA only).

Transfection Resource: <u>altogen.com/transfection-resource</u>

Altogen Labs. GLP Compliant Pre-clinical CRO Laboratory Services:

AltogenLabs.com

Recommended Transfection Protocols (for 24-well plate):

Beta-TC6 Standard Transfection Protocol (24-well plate):

- **1.** Plate 10,000 15,000 Beta-TC6 cells per well in 0.5 ml of complete growth medium 12–24 hours prior to transfection
- 2. Wash with 1xPBS and add 0.5 ml of fresh growth medium
- 3. Prepare transfection complexes by mixing 40 μl of serum-free medium, 5.5 μl of transfection reagent, and
 - 750 ng DNA (or mRNA), or
 - 30 nM 50 nM of siRNA (or microRNA)
 - *Referred to a final volume including growth medium
- **4.** Incubate transfection complexes at RT for 15 30 minutes
- **5.** Optional: Add $2 \mu l$ of Complex Condenser. This reagent reduces the size of transfection complex, therefore increasing transfection efficiency; however it may increase cell toxicity
- **6.** Add prepared transfection complexes to 0.5 ml of complete growth medium with Beta-TC6 cells (from step 2)
- 7. Incubate cells at 37° C in a humidified CO_2 incubator
- **8.** Assay for phenotype or target gene expression 48 72 hours after transfection

Optional: Transfection efficiency can be increased by addition of Transfection Enhancer reagent. Add 2 μl of Transfection Enhancer reagent 12-24 hours after transfection If the viability of Beta-TC6 cells being transfected is affected at 16 - 24 hours post-transfection, the level of cytotoxicity can be decreased by changing the growth medium and eliminating redundant exposure of cells to transfectant

Beta-TC6 Reverse Transfection Protocol (24-well plate):

- **1.** Prepare Beta-TC6 cell suspension:
 - a. Trypsinize cells (0.05% Trypsin) for 3-5 minutes at 37°C
 - **b.** Dilute in complete growth medium to 5 x 10⁴ cells/ml
- **2.** Prepare transfection complexes by mixing 40 μ l of serum-free medium, 5.5 μ l of transfection reagent, and
 - 750 ng DNA (or mRNA), or
 - 30 nM 50 nM of siRNA (or microRNA)
 - *Referred to a final volume including growth medium
- 3. Incubate transfection complexes at RT for 15 30 minutes
- **4.** Optional: Add 2 μ l of Complex Condenser. This reagent reduces the size of transfection complex, therefore increasing transfection efficiency; however it may increase cell toxicity
- **5.** Plate 20,000 30,000 cells per well in 0.5 ml of complete growth medium (from step #1) into culture plate
- **6.** Add prepared transfection complexes (from step 3 or 4)
- 7. Incubate cells at 37°C in a humidified CO₂ incubator
- **8.** Assay for phenotype or target gene expression 48 72 hours after transfection

Optional: Transfection efficiency can be increased by addition of Transfection Enhancer reagent. Add 2 μl of Transfection Enhancer reagent 12-24 hours after transfection If the viability of Beta-TC6 cells being transfected is affected at 16 - 24 hours post-transfection, the level of cytotoxicity can be decreased by changing the growth medium and eliminating redundant exposure of cells to transfectant

Scaling Up or Down Transfections:

Culture Vessel Surface Area (cm ²)	Volume of Growth Medium (ml)	Transfection Reagent (µl)	Complex Condenser (µl)	Transfection Enhancer (μl)
96-well, 0.3 cm x cm	0.12	1.5	0.3	0.3
24-well, 2 cm x cm	0.5	5.5	2	2
12-well, 4 cm x cm	1	12	4	4
6-well, 10 cm x cm	3	35	12	12
60-mm, 20 cm x cm	5	60	20	20
10-cm. 60 cm x cm	15	180	60	60

Optimizing Transfection:

To obtain the highest transfection efficiency, optimize transfection conditions by varying Beta-TC6 cell density and amount of transfection reagent. High passage of Beta-TC6 cells and use of antibiotics (or growth factors) may require using larger volumes of Beta-TC6 transfection reagent per reaction.

Limited Use Label License:

The purchase of this product conveys to the purchaser the limited right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. This product is for research purposes only and is not for use in commercial applications of any kind. For information on obtaining additional rights, please contact Altogen Biosystems at orders@altogen.com.

Limited Product Warranty:

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