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DETROIT551 Transfection Protocol (for Embryonic Fibroblast Cells, CCL-110)

Catalog No. 6801 Size: 0.5 ml
Catalog No. 6802 Size: 1.5 ml
Catalog No. 7035 Size: 8.0 ml

Contents and Shipping:
DETROIT551 Transfection Kit includes DETROIT551 Transfection Reagent (0.5 ml / 1.5 ml / 8.0 ml), Transfection Enhancer (0.5 ml), and Complex Condenser (0.5 ml). DETROIT551 Transfection Reagent is supplied in liquid form at a concentration of 0.25 mg/ml, shipped at ambient temperature.

Description:
Polymer-based transfection reagent is a proprietary formulation optimized for transfection of DNA and RNA into DETROIT551 cells.

Product Qualification:
DETROIT551 Transfection Reagent is tested functionally by transfection of DETROIT551 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 4ºC 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: altogen.com/transfection-resource

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

Recommended Transfection Protocols (for 24-well plate):

DETROIT551 Standard Transfection Protocol (24-well plate):	DETROIT551 Reverse Transfection Protocol (24-well plate):
1. Plate 10,000 - 15,000 DETROIT551 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, 4.5 µl of transfection reagent, and • 500 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 µl 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 DETROIT551 cells (from step 2) **7.** Incubate cells at 37ºC in a humidified CO_2 incubator **8.** Assay for phenotype or target gene expression 48 - 72 hours after transfection	**1.** Prepare DETROIT551 cell suspension: **a.** Trypsinize cells (0.05% Trypsin) for 3-5 minutes at 37°C **b.** Dilute in complete growth medium to 5 x 10^4 cells/ml **2.** Prepare transfection complexes by mixing 40 µl of serum-free medium, 4.5 µl of transfection reagent, and • 500 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 µl 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 37ºC 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	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 DETROIT551 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	If the viability of DETROIT551 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	1.2	0.3	0.3
24-well, 2 cm x cm	0.5	4.5	2	2
12-well, 4 cm x cm	1	10	4	4
6-well, 10 cm x cm	3	30	12	12
60-mm, 20 cm x cm	5	50	20	20
10-cm, 60 cm x cm	15	150	60	60

Optimizing Transfection:

To obtain the highest transfection efficiency, optimize transfection conditions by varying DETROIT551 cell density and amount of transfection reagent. High passage of DETROIT551 cells and use of antibiotics (or growth factors) may require using larger volumes of DETROIT551 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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