DITNC1 Transfection Kit (for Rat Brain Astrocyte Cells)

Catalog No. 1747  Size: 0.5 ml
Catalog No. 1748  Size: 1.5 ml
Catalog No. 7036  Size: 8.0 ml

Contents and Shipping:
DITNC1 Transfection Kit includes DITNC1 Transfection Reagent (0.5 ml / 1.5 ml / 8.0 ml), Transfection Enhancer (0.5 ml), and Complex Condenser (0.5 ml). DITNC1 Transfection Reagent is supplied in liquid form at a concentration of 0.35 mg/ml, shipped at ambient temperature.

Description:
Nanoparticle-based liposome transfection reagent is a proprietary formulation optimized for transfection of DNA and RNA into DITNC1 cells.

Product Qualification:
DITNC1 Transfection Reagent is tested functionally by transfection of DITNC1 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 6 months.

Intended Use:
For *in vitro* use only.

MSDS:
MSDS documents are available online at www.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 µg)
- 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 (www.altogen.com) using credit card payment. Purchase Order (PO) can be faxed at (702) 989-0841 (for USA only).

Transfection Resource:  www.altogen.com/transfection-resource

Altogen Labs. GLP Compliant Pre-clinical CRO Laboratory Services:  www.altogenlabs.com
DITNC1 Standard Transfection Protocol (24-well plate):

1. Plate 15,000 - 20,000 DITNC1 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 µl of transfection reagent, and:
   - 500 ng DNA (or mRNA), or
   - 30 nM - 50 nM of siRNA (or microRNA)
*Referenced 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 DITNC1 cells (from step 2).
7. Incubate cells at 37°C in a humidified CO2 incubator.
8. Assay for phenotype or target gene expression 48 - 72 hours after transfection.

DITNC1 Reverse Transfection Protocol (24-well plate):

1. Prepare DITNC1 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, 5 µl of transfection reagent, and:
   - 500 ng DNA (or mRNA), or
   - 30 nM - 50 nM of siRNA (or microRNA)
*Referenced 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 25,000 - 35,000 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 DITNC1 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:

<table>
<thead>
<tr>
<th>Culture Vessel</th>
<th>Volume of Growth Medium (ml)</th>
<th>Transfection Reagent (µl)</th>
<th>Complex Condenser (µl)</th>
<th>Transfection Enhancer (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well, 0.3 cm x cm</td>
<td>0.12</td>
<td>1.4</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>24-well, 2 cm x cm</td>
<td>0.5</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>12-well, 4 cm x cm</td>
<td>1</td>
<td>11</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>6-well, 10 cm x cm</td>
<td>3</td>
<td>32</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>60-mm, 20 cm x cm</td>
<td>5</td>
<td>55</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>10-cm, 60 cm x cm</td>
<td>15</td>
<td>160</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

Optimizing Transfection:
To obtain the highest transfection efficiency, optimize transfection conditions by varying DITNC1 cell density and amount of transfection reagent. High passage of DITNC1 cells and use of antibiotics (or growth factors) may require using larger volumes of DITNC1 transfection reagent per reaction.

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