AltoFect Transfection Protocol (kit for primary cell types)

Catalog No. 3001  Size: 0.5 ml
Catalog No. 3002  Size: 1.5 ml
Catalog No. 3003  Size: 8.0 ml

Contents and Shipping:
AltoFect Transfection Kit includes AltoFect™ Transfection Reagent (0.5 ml / 1.5 ml / 8.0 ml), Transfection Enhancer (0.5 ml), and Complex Condenser (0.5 ml). AltoFect™ Transfection Reagent is supplied in liquid form at a concentration of 1 mg/ml, shipped at ambient temperature.

Description:
Two component liposome-based proprietary formulation optimized for primary cell types and difficult-to-transfect cancer cell lines.

Product Qualification:
AltoFect™ Transfection Reagent is tested functionally by transfection of primary mouse fibroblast cells with a small interfering RNAs targeting 3 different genes (Lamin A/C, GAPDH, Cyclophilin B). 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 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 (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):

**AltoFect Standard Transfection Protocol (24-well plate):**

1. Plate 10,000 - 15,000 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, 2 µl of transfection reagent, and
   - 1 µg DNA (or mRNA), or
   - 30 nM of siRNA (or microRNA)
   *Referred to a final volume including growth medium
4. Incubate transfection complexes at RT for 15 - 20 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 cultured 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

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 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 transfection complexes

**AltoFect Reverse Transfection Protocol (24-well plate):**

1. Prepare 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 µl of serum-free medium, 2 µl of transfection reagent, and
   - 1 µg DNA (or mRNA), or
   - 30 nM of siRNA (or microRNA)
   *Referred to a final volume including growth medium
3. Incubate transfection complexes at RT for 15 - 20 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 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 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 transfection complexes

### 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>0.7</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>24-well, 2 cm x cm</td>
<td>0.5</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>12-well, 4 cm x cm</td>
<td>1</td>
<td>4.5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>6-well, 10 cm x cm</td>
<td>3</td>
<td>14</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>60-mm, 20 cm x cm</td>
<td>5</td>
<td>25</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>10-cm, 60 cm x cm</td>
<td>15</td>
<td>70</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

### Optimizing Transfection:

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

### Limited Use Label License:

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