IRR-MRC5 Transfection Kit (for Irradiated Fibroblast Cells, 55-X)

Catalog No. 6833  Size: 0.5 ml
Catalog No. 6834  Size: 1.5 ml
Catalog No. 7050  Size: 8.0 ml

Contents and Shipping:
IRR-MRC5 Transfection Kit includes IRR-MRC5 Transfection Reagent (0.5 ml / 1.5 ml / 8.0 ml), Transfection Enhancer (0.5 ml), and Complex Condenser (0.5 ml). IRR-MRC5 Transfection Reagent is supplied in liquid form at a concentration of 0.25 mg/ml, shipped at ambient temperature.

Description:
Cationic lipid based reagent is a proprietary formulation optimized for transfection of DNA and RNA into IRR-MRC5 cells.

Product Qualification:
IRR-MRC5 Transfection Reagent is tested functionally by transfection of IRR-MRC5 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 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 (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
Recommended Transfection Protocols (for 24-well plate):

**IRR-MRC5 Standard Transfection Protocol (24-well plate):**

1. Plate 7,500 - 12,000 IRR-MRC5 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 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 IRR-MRC5 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.

**IRR-MRC5 Reverse Transfection Protocol (24-well plate):**

1. Prepare IRR-MRC5 cell suspension:
   - Trypsinize cells (0.05% Trypsin) for 3-5 minutes at 37°C.
   - Dilute in complete growth medium to 5 x 10^4 cells/ml.
2. Prepare transfection complexes by mixing 40 µl of serum-free medium, 5.5 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
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 15,000 - 25,000 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 IRR-MRC5 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 Surface Area (cm²)</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>1.5</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>24-well, 2 cm x cm</td>
<td>0.5</td>
<td>5.5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>12-well, 4 cm x cm</td>
<td>1</td>
<td>12</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>6-well, 10 cm x cm</td>
<td>3</td>
<td>35</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>60-mm, 20 cm x cm</td>
<td>5</td>
<td>60</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>10-cm, 60 cm x cm</td>
<td>15</td>
<td>180</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

### Optimizing Transfection:

To obtain the highest transfection efficiency, optimize transfection conditions by varying IRR-MRC5 cell density and amount of transfection reagent. High passage of IRR-MRC5 cells and use of antibiotics (or growth factors) may require using larger volumes of IRR-MRC5 transfection reagent per reaction.

### Limited Use Label License:

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