**DNA-In™ Transfection Reagent  “Quick Start Protocol”**

## Storage & Stability
- DNA-In™ Reagent is shipped at room temperature. Store at 4°C. DO NOT FREEZE!

## Materials Required
- DNA-In™ Reagent
- Opti-MEM® 1 Reduced Serum Medium (*not supplied*)
- Plasmid DNA reporter (*not supplied*)

## Overview
DNA-In™ Reagent is a formulation of chemically defined compounds, and is completely free of animal-derived components. The protocol provided below has been optimized to achieve the highest number of cells transfected in a population. It is recommended that the first set of experiments be done using a GFP reporter system to optimize percent cells transfected with DNA-In™ Reagent. The amount of plasmid DNA/DNA-In™ Reagent complex that is added to cells is a critical factor in determining percent cells transfected, level of expression, and cellular toxicity. This reagent has been optimized for intracellular delivery of DNA into cultured mammalian cells in the presence of serum at a cell density of 70% to 90%. For most cell lines, high levels of expression can be achieved using the amount of DNA-In™ Reagent and DNA recommended in the following protocol.

For best results, it is important to empirically determine the optimal amount of DNA and DNA-In™ Reagent for any given cell line. **If toxicity is observed, reducing the amount of DNA may reduce toxicity** while still maintaining high levels of expression.

## Important Notes Before You Start
- **Antibiotics** - Do not add antibiotics to medium during transfection as this leads to cell death.
- **Transfection Optimization** - The optimal concentrations of DNA-In™ transfection reagent and DNA should be determined empirically for each cell line (*see section Optimization and Scaling*).
- **DNA Concentrations** - Cytotoxicity is greatly influenced by the amount of DNA present; the lowest concentration which provides adequate expression should be used.

## Transfection Protocol
This protocol is written for transfection of cells in a 24-well plating format. It may be adapted to other formats by scaling the volumes up or down to fit the format used (see table next page).

### Day Before Transfection:
Approximately 24 hours before transfection, cells should be plated such that the cell density is approximately 70-80% confluent at the time of transfection in complete medium without antibiotics. For a 24-well plate format, cells should be plated in 500µl of medium per well.

### Day of Transfection - Initial Preparations:
1. Thaw DNA at room temperature.
2. Allow the DNA-In™ Transfection Reagent to reach room temperature.
3. Mix the reagent by inversion of the tube several times.

- Add 1µg DNA/100µl OptiMEM
- Add 3µl DNA-In™
- Vortex 2-3s, Incubate @RT 10-15 min
- Add complex drop wise to multiple regions of designated wells of cultured cells. Swirl gently to mix.
- Add 50µl, 25µl & 12.5µl volumes
- Incubate cells @37°C. Observe reporter gene expression 24-48hr. post-transfection.
General Transfection Procedure:
1. To a sterile tube (or U-bottom plate) add 100µl of Opti-MEM medium pre-warmed to room temperature. Add 1µg (typically 1µl) of DNA to be transfected to medium containing tube and mix. Add 3µl of DNA-In™ Transfection Reagent to the diluted DNA solution, mix well.
2. Incubate the complexing mixture at room temperature for 10-15 minutes.
3. To each of 3 cell-containing wells, add 12.5µl, 25µl, or 50µl* of the complexing reaction to the 500µl of existing medium.
4. Mix thoroughly, but gently. Return cells to incubator.

5. After an appropriate length of incubation, typically 24-48 hours, measure the transfection efficiency via an assay tailored to the reporter gene that was used.

* 50 µl of complex is equivalent to 1.5 µl DNA-In™ and 500 ng DNA.

Optimization and Scaling

Do not make DNA/DNA-In™ Reagent complexes in volumes smaller than 20µl nor handle individual components in volumes of less than 1 µl. DNA-In™ Reagent may be diluted in Opti-MEM®I immediately before use, if needed. Diluted reagent is not stable to storage and should be discarded.

Table 1 - Recommended quantities for transfecting DNA in various plate formats.

<table>
<thead>
<tr>
<th>Culture plate</th>
<th>Relative surface area (cm²/well)</th>
<th>Volume of complete medium/well</th>
<th>Volume of DNA/DNA-In™ complex/well</th>
<th>Recommended starting DNA amount</th>
<th>Range of DNA/well for optimization</th>
<th>Amount of DNA-In™ Reagent/ well</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well</td>
<td>0.2x</td>
<td>100µl</td>
<td>10µl</td>
<td>0.1µg</td>
<td>0.05-0.2µg</td>
<td>0.1-0.6µl</td>
</tr>
<tr>
<td>48-well</td>
<td>0.4x</td>
<td>200µl</td>
<td>20µl</td>
<td>0.2µg</td>
<td>0.1-0.4µg</td>
<td>0.2-1.2µl</td>
</tr>
<tr>
<td>24-well</td>
<td>1x</td>
<td>500µl</td>
<td>50µl</td>
<td>0.5µg</td>
<td>0.2-1.0µg</td>
<td>0.5-3µl</td>
</tr>
<tr>
<td>6-well</td>
<td>5x</td>
<td>2.5ml</td>
<td>250µl</td>
<td>2.5µg</td>
<td>1-5µg</td>
<td>2.5-15µl</td>
</tr>
</tbody>
</table>

The amount of DNA used in forming transfection complexes determines toxicity. Optimization involves determining the optimal amount of DNA along with the best reagent to DNA ratio. Generally, as a starting point, MTI recommends examining at least four (4) different DNA amounts over an 8- to 10-fold range matrixed with DNA-In™ Reagent over a 4-fold range. For example, in a 24-well format, we suggest setting up complexing reactions with 0.125, 0.25, 0.5 and 1.0 µg DNA per 50 µl of serum-free medium. For each DNA amount, add 1, 2, 3, or 4 µl of DNA-In™ Reagent. As controls, include ‘Reagent alone’ and ‘DNA alone’ added to cell-containing wells.

Results from the preceding “Quick Start Protocol” may help guide the optimization process. A good starting point may be the low, middle, or high portions of the preceding recommended ranges and amounts depending on whether best results were observed with the 12.5µl, 25µl, or 50µl transfections, respectively. I.e., if the best result was obtained with the 25 µl addition, this would indicate that optimization experiments should center on 0.25 µg DNA/50 µl of complex with this particular cell type. For a more complete discussion of this topic, please visit our web site at the address below.

For more detailed instructions, other tips and troubleshooting, please visit our Web site at www.moleculartransfer.com or visit Molecular Transfer Inc webstore at www.vitascientific.com for more information.

1 Opti-MEM®I is a registered trademark of Life Technologies Corp.