# DNA-In™

Transfection Reagent

"Quick Start Protocol"

## Overview

**DNA-In™ Transfection 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 (%CT), without toxicity**. Higher expression levels can be obtained later by addition of more DNA if required. 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 in the presence of serum at a cell density of 70% to 90%. 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 and % cell transfected. If using lower confluency cells reduce volumes of DNA-In™ Reagent and DNA.

## Storage & Stability

- DNA-In™ Transfection Reagent is shipped at room temperature. Store at 4°C. DO NOT FREEZE!

## Materials Required

- DNA-In™ Transfection Reagent
- Opti-MEM® I[1] Reduced Serum Medium ( not supplied)
- pMTICAG-GFP (10µg /ml in sterile tissue culture grade PBS without $Mg^{2+}$/ $Ca^{2+}$) supplied in 0.1ml SKU

## 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 cells (*see section Optimization and Scaling*).
- **DNA Concentrations** - Cytotoxicity is greatly influenced by the amount of DNA present and the optimal amount should be determined for cells. The lowest concentration which provides adequate expression should be used. **If toxicity is observed, reducing the amount of DNA may reduce toxicity** while still maintaining high levels of expression.

## *Transfection Protocol – Quick Start*

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).

### *A. Day Before Transfection - Cell Plating Preparation*

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.

### *B. Day of Transfection – Reagent Preparation:*

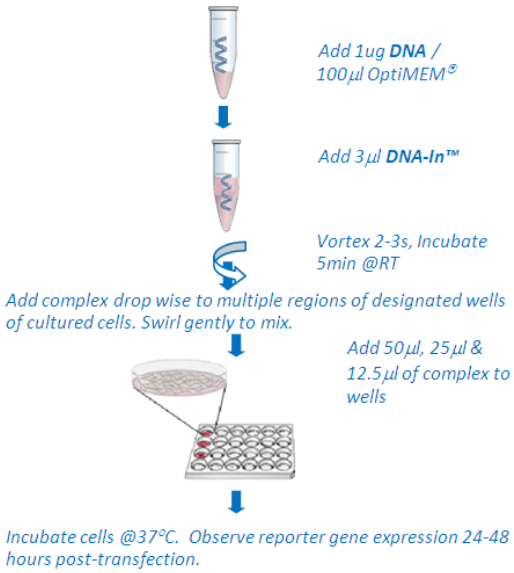
1. Thaw DNA at room temperature.
2. Allow the **DNA-In™ Transfection Reagent** to reach room temperature.
3. Mix the reagent by inversion of the tube several times.

### *C. Day of Transfection – General Transfection Protocol*

4. To a sterile tube add 100µl of provided DNA or add 100µl of Opti-MEM®I medium pre-warmed to room temperature. Add 1µg (typically 1µl) of DNA to be transfected to medium containing tube and mix.
5. Add 3µl of DNA-In™ Transfection Reagent to the diluted DNA solution, mix well.

*TransfectionProtcol Schematic*

***** Use 100µl of supplied DNA for the first experiment***




### *C. General Protocol Continued…*

6. Incubate the complexing mixture at room temperature for 10-15 minutes.

7. To each of 3 cell-containing wells, add 12.5µl, 25µl, or 50µl* of the complexing reaction to the 500µl of existing medium.
8. Mix thoroughly, but gently. Return cells to incubator.
9. 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™ Transfection Reagent and 500 ng DNA.*

## *Optimization and Scale-Up*

Results from the above "Quick Start Protocol" will help guide the optimization process. If the best result was obtained with the 25µl addition of complex (250ng DNA) this would indicate that optimization experiments should center on 0.25µg DNA per 50µl of Opti-MEM with this particular cell type. Titrate various amounts of the DNA-In™ Reagent with 0.250µg of DNA. Different DNA constructs may require more or less DNA depending on function. If toxicity was observed with the 12.5µl addition, reduce the amount DNA-In™. If no toxicity was observed with the 50µl of complex then adding more DNA and DNA-In™ can be consider to increase expression or for higher % cells transfected.

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

| Culture Plate | Relative Surface Area ($cm^2$/well) | Volume of Complete Medium | Volume of DNA / DNA-In™ Complex | Recommended *Start Volume* of DNA | Volume range of DNA for *Optimization* | Volume of DNA-In™ / well |
|---|---|---|---|---|---|---|
| 96-well | 0.2x | 100µl | 10µl | 0.1µg | 0.05-0.2µg | 0.1-0.6µl |
| 48-well | 0.4x | 200µl | 20µl | 0.2µg | 0.1-0.4µg | 0.2-1.2µl |
| 24-well | 1x | 500µl | 50µl | 0.5µg | 0.2-1.0µg | 0.5-3µl |
| 6-well | 5x | 2.5ml | 250µl | 2.5µg | 1-5µg | 2.5-15µl |

The amount of DNA used in forming transfection complexes determines toxicity for a given cell type. Optimization involves determining the optimal amount of DNA along with the best reagent to DNA ratio. Generally, as a starting point we recommend examining at least **four (4) different DNA amounts** over an 8 to 10-fold range **matrixed with DNA-In™ Transfection 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 0.5, 1.0, 1.5, and 2.0 µl of DNA-In Transfection Reagent**. As controls, include 'Reagent alone' and 'DNA alone' added to cell-containing wells.

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



AMSBIO| www.amsbio.com | info@amsbio.com

**UK & Rest of the World**
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

**North America**
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

**Germany**
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

**Switzerland**
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85