amsbio MTI-GlobalStem

DNA-In™*HepG2*

Transfection Reagent "Quick Start Protocol"

Overview

DNA-In™ *HepG2* **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. It is recommended that the first set of experiments be done using a GFP reporter system to optimize percent cells transfected with DNA-In™ *HepG2* Transfection Reagent. The amount of plasmid DNA/ DNA-In™ *HepG2* Transfection 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 HepG2 cells in the presence of serum at a cell density of 70% to 90%. For this cell line, high levels of expression can be achieved using the amount of DNA-In™ *HepG2* Transfection Reagent and DNA recommended in the following protocol.

Storage & Stability

- DNA-In™ *HepG2* Transfection Reagent is shipped at room temperature. Store at 4°C. DO NOT FREEZE!

Materials Supplied

- DNA-In™ *HepG2* Transfection Reagent

Materials required but not supplied

- Opti-MEM® I[1] Reduced Serum Medium
- Plasmid DNA reporter

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™ *HepG2* Transfection Reagent and DNA should be determined empirically for HepG2 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 HepG2 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

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.

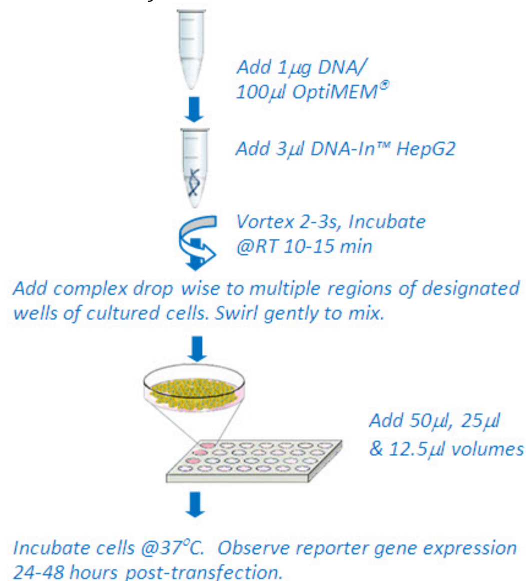
B. Day of Transfection – Transfection Reagent Preparation:

1. Thaw DNA at room temperature.
2. Allow the **DNA-In™ *HepG2* 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 (or U-bottom plate) 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. Add 3µl of DNA-In™ *HepG2* Transfection Reagent to the diluted DNA solution, mix well.

TransfectionProtcol Schematic



C. General Protocol Continued…

5. Incubate the complexing mixture at room temperature for 10-15 minutes.
6. To each of 3 cell-containing wells, add 12.5µl, 25µl, or 50µl* of the complexing reaction to the 500µl of existing medium.
7. Mix thoroughly, but gently. Return cells to incubator.
8. 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™ HepG2 Transfection Reagent and 500 ng DNA.*

Optimization and Scale-Up

Do not make DNA/DNA-In™ *HepG2* Transfection Reagent complexes in volumes smaller than 20µl nor handle individual components in volumes of less than 1µl. DNA-In™ *HepG2* Transfection 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.

Culture Plate	Relative Surface Area (cm²/well)	Volume of Complete Medium	Volume of DNA / DNA-In™ *HepG2* Complex	Recommended *Start Volume* of DNA	Volume of DNA for *Optimization*	Volume of DNA-In™ *HepG2*
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. 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™ *HepG2* 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 1, 2, 3, or 4 µl of DNA-In™ *HepG2* Transfection Reagent**. As controls, include 'Reagent alone' and 'DNA alone' added to cell-containing wells.

Results from this "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 25µl addition, this would indicate that optimization experiments should center on 0.25 µg DNA per 50µl of complex with this particular cell type. For a more complete discussion of this topic, please visit our web site at the address below.

Notice to Buyer: Limited Label License

Use of DNA-In™ Transfection Reagent is covered by U.S. Patent No. 6,150,168 and applications.

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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