GlobalStem®
We Take Our Cells Seriously™

GeneIn™
Transfection Reagent

INSTRUCTIONS FOR USE *For Research Use Only*

I. STORAGE & STABILITY

- **GeneIn™** Reagent is shipped on wet ice.
- Store at 4ºC upon receipt. ***Do not freeze***.
- Guaranteed to be stable for 6 months from date of purchase, when properly stored & handled.

II. MATERIALS

GeneIn™ Reagent is supplied in ***one*** of the following formats.

Catalog No.	Size
GST-1000	0.25 ml
GST-1001	0.75 ml
GST-1002	1.5 ml
GST-1003	5 x 1.5 ml
GST-1004	15 ml

III. OVERVIEW

GeneIn™ Reagents are a formulation of proprietary compounds that are chemically defined and are of animal-free origin. The protocols provided below have 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 GeneIn™ Reagents. The amount of DNA/GeneIn™ Reagent complex that is added to cells is a critical factor in determining percent cells transfected, level of expression and cellular toxicity. These reagents have 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 recommended amount of GeneIn™ Reagents and DNA in this manual. GeneIn™ has not been optimized for RNA delivery. For best results, it is important to empirically determine the optimal amount of DNA and GeneIn™ Reagents for any given cell line.

IV. GETTING STARTED

To start, follow the GeneIn™ Start Protocol - Rapid 24-Well Plate Optimization as outlined on page 2. The 24-Well Plate optimization protocol gives instruction for determining the optimal GeneIn™ Reagents and DNA concentrations for your specific cell type. Review carefully the complete protocol before beginning. Once the concentrations are determined using the Rapid 24-Well Plate Optimization Protocol, use the *Fine Tune* Protocol *(p. 5)* to further optimize and improve your transfection by two fold or greater.

India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA). Ph: +91-11-42208000, 42208111 Mobile: +91-9810521400
Fax: +91-11-42208444 Email: customerservice@lifetechindia.com Website: www.lifetechindia.com

GeneIn™ *START PROTOCOL – Rapid 24-Well Plate Optimization*

Review carefully the complete protocol before beginning.

DAY 1 - PRE-TRANSFECTION

1. The day before transfection, inoculate a 24-well tissue culture plate with an appropriate number of cells in serum containing growth medium (*that does not contain antibiotics*) such that cells will be 70-80% confluent the following day. ***(See Table 1 for recommended reagent volumes per well for different plate formats).***
2. Incubate the cells overnight under desired conditions. Suspension cells can be optimized using this protocol but may require higher DNA concentrations, depending on cell density.

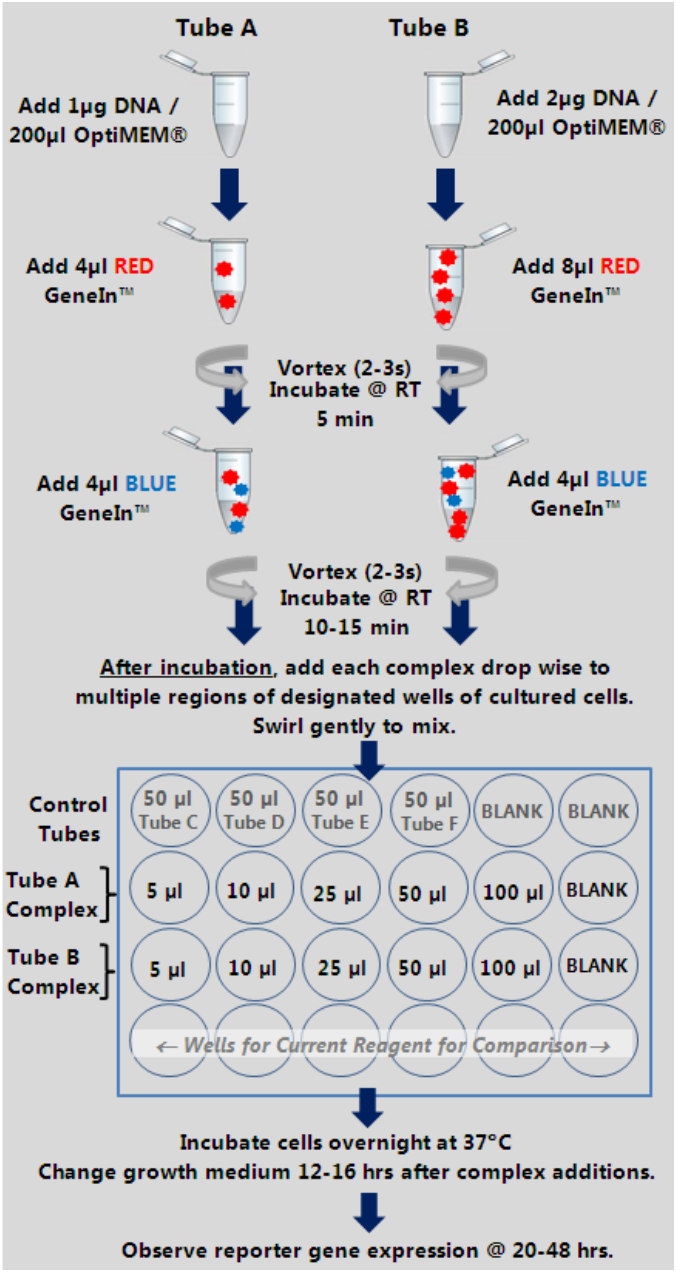
DAY 2 - TRANSFECTION

1. Bring reagents to room temperature before starting.
2. Add 200µl of serum-free medium (OptiMEM®I Reduced Serum Medium) into two different 1.5ml polypropylene tubes(Tube A and Tube B) *NB: OptiMEM is product of Invitrogen*
3. Add 1µg of DNA to Tube A and 2µg of DNA to Tube B.
4. **Complex Formulation:** Mix each GeneIn™ Reagent prior to preparing complexes. Add 4µl of **RED GeneIn™** Reagent to **Tube A** containing 200µl of DNA solution (5µg/ml). Vortex for 2-3 seconds. Add 8µl of **RED GeneIn™** Reagent to **Tube B** (10µg/ml). Vortex for 2-3 seconds. Incubate 5 minutes at room temperature. Immediately add 4µl of **BLUE GeneIn™** Reagent to each tube and vortex for 2-3 seconds. **Incubate each tube for 10-15 minutes at room temperature.**
5. **After incubation**, add 5, 10, 25, 50, and 100µl of complexes dropwise directly to the corresponding wells of the inoculated 24-well cell culture plate (See Figure 1). Swirl plate gently.
6. **Negative Controls**: Prepare controls by setting up 4 separate tubes (C, D E & F) that contain the following:

Tube C	1µl of **RED GeneIn™** Reagent / 100µl of OptiMEM® I
Tube D	1µl of **BLUE GeneIn™** Reagent / 100µl of OptiMEM® I
Tube E	1µl of Plasmid DNA / 100µl of Opti-MEM® I
Tube F	100µl of Opti-MEM® I Only

7. Mix each control tube briefly, and add 50µl of these mixes to individual wells of cultured cells on the plate as illustrated (see Figure 1. *24-Well Plate Schematic).* Incubate the cells at 37°C in a CO_2 incubator
8. Change growth medium 12-16 hours after addition of complex
9. Expression of reporter gene activity should generally be assessed at 20-48 hours post-transfection using an appropriate method. GFP expression is maximal at 40-48 hours post-transfection.

FIGURE 1.



India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA). Ph: +91-11-42208000, 42208111 Mobile: +91-9810521400
Fax: +91-11-42208444 Email: customerservice@lifetechindia.com Website: www.lifetechindia.com

TABLE 1: Recommended Reagent Quantities for Different Plate Configurations:

	Cell Culture Plate	Volume of Culture Medium/Well	Plasmid DNA Solution @ 5 µg/ml (Tube A)	Volume of RED GeneIn™ for 5 µg/ml of Plasmid DNA Solution (Tube A)	Plasmid DNA Solution @ 10 µg/ml (Tube B)	Volume of RED GeneIn™ For 10 µg/ml of Plasmid DNA Solution (Tube B)	Volume of BLUE GeneIn™ (Tubes A & B)	Volume of DNA/GeneIn™ Reagent Complex Added to the Cells (µl)
	96-well	0.1ml	100µl	2µl	100µl	4µl	2µl	1, 2.5**, 5, 10,** 20 µl
*	24-well	0.5ml	200µl	4µl	200µl	8µl	4µl	5, 10, **25, 50,** 100 µl
	12-well	1ml	400µl	8µl	400µl	16µl	8µl	12.5, 25, **50, 100,** 200 µl
	6-well	2ml	800µl	16µl	800µl	32µl	16µl	25, 50, **100, 200,** 400 µl
	60-mm	5ml	2000µl	40µl	2000µl	80µl	40µl	50, 100, **250, 500,** 1000 µl
	100-mm	10ml	4000µl	80µl	4000µl	160µl	80µl	100, 200, **500, 1000,** 2000 µl

Recommended start volumes shown in bold

V. RECOMMENDATIONS FOR BEST RESULTS

- Mix each tube of GeneIn™ Reagent by inversion before each use.
- **Start optimization with 5µg/ml and 10µg/ml DNA solutions.**
- GeneIn™ Reagents are designed to work in serum but can be used under serum-free or reduced serum conditions with proper optimization. It may be necessary to reduce the amount of reagents when using serum-free or reduced serum medium.
- **Do not** use antibiotics in the transfection medium and avoid using antibiotics when plating cells for transfection.
- Use of untreated sterile polystyrene plastic-ware (multi-well plates with U or V-bottoms) is preferred when preparing the DNA/GeneIn™ Reagent complex for 96-well plate transfections.
- Sterile polypropylene tubes (such as Eppendorf tubes) can be used to make larger amounts of DNA/GeneIn™ Reagent complex.
- **Do not** make DNA/GeneIn™ Reagent complex in less than a total volume of 20µl. To avoid complexing reaction volumes of less than 20µl, the RED and BLUE GeneIn™ Reagents may be diluted in [1]Opti-MEM® I Reduced Serum Medium just prior to use. The diluted reagents are not stable and should be discarded.

India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA). Ph: +91-11-42208000, 42208111 Mobile: +91-9810521400
Fax: +91-11-42208444 Email: customerservice@lifetechindia.com Website: www.lifetechindia.com

VI. OPTIMIZATION OVERVIEW

Determining the optimal conditions for transfection efficiency (% Cells Transfected) is essential for achieving high levels of gene expression with minimal levels of cellular toxicity. In general, gene expression will increase, plateau and then decrease with increasing amounts of DNA/GeneIn™ Reagent complex.

The amount of DNA used directly affects expression level and toxicity. The % cells transfected may be the same with high or low levels of DNA but toxicity may be dramatically different. The optimization protocol recommends the use of two different concentrations of DNA for making the complex as a starting point. Reducing the amount of DNA when preparing the transfection complexes, along with the amount of GeneIn™ Reagents, can reduce toxicity. The amount of plasmid DNA required for maximal expression of a given reporter gene can vary from one cell line to another.

NOTE: The critical parameter to optimize for any given culture vessel and cell density is the amount of nucleic acid/GeneIn™ Reagent complex added to the cells. **See Table 1 for recommended starting reagent amounts and DNA concentrations for different culture plate/format sizes.**

India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA). Ph: +91-11-42208000, 42208111 Mobile: +91-9810521400
Fax: +91-11-42208444 Email: customerservice@lifetechindia.com Website: www.lifetechindia.com

GeneIn™ *FINE-TUNE PROTOCOL (OPTIONAL)*

Once the optimal DNA concentration is determined using the Rapid 24-Well Plate Optimization Protocol, further optimization of the **RED GeneIn™** and/or **BLUE GeneIn™** reagent may be performed to improve transfection two fold or more.

NOTE: The following example (Table 2) assumes that the 5µg/ml DNA complex reactions gave the best overall transfection profile.

TABLE 2: RECOMMENDED REAGENT QUANTITIES FOR FINE TUNING IN A 24-WELL PLATE:

Tube	Volume of Culture Medium/well	Plasmid DNA Solution @ 5µg/ml *	Volume of RED GeneIn™ Reagent	Volume of BLUE GeneIn™ Reagent	Volume of DNA/GeneIn™ Reagent Complex Added to the Cells (µl)
A	0.5ml	100µl	2µl	1µl	12.5, 25, 50µl
B	0.5ml	100µl	2µl	2µl	12.5, 25, 50µl
C	0.5ml	100µl	2µl	3µl	12.5, 25, 50µl
D	0.5ml	100µl	2µl	4µl	12.5, 25, 50µl
E	0.5ml	100µl	4µl	1µl	12.5, 25, 50µl
F	0.5ml	100µl	4µl	2µl	12.5, 25, 50µl
G	0.5ml	100µl	4µl	3µl	12.5, 25, 50µl
H	0.5ml	100µl	4µl	4µl	12.5, 25, 50µl

*** Actual amount of DNA solution used here should be based on the optimal DNA concentration determined in the *START PROTOCOL.***

FIGURE 2: FINE-TUNING LAYOUT FOR A 24-WELL PLATE:

	(1) 12.5 µl Complex	(2) 25 µl Complex	(3) 50 µl Complex	(4) 12.5 µl Complex	(5) 25 µl Complex	(6) 50 µl Complex
A	Tube A	Tube A	Tube A	Tube B	Tube B	Tube B
B	Tube C	Tube C	Tube C	Tube D	Tube D	Tube D
C	Tube E	Tube E	Tube E	Tube F	Tube F	Tube F
D	Tube G	Tube G	Tube G	Tube H	Tube H	Tube H

EXPRESSION LEVEL TITRATION: DNA amounts can be increased or decreased depending on the level of expression desired. For example, if 5µg/ml DNA concentration shows levels of expression higher than desired, reduce the amount of DNA. If higher levels of expression are desired, use a higher DNA concentration (*e.g.* 20µg/ml) to make complexes and titrate the amount of complex added to cells.

India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA). Ph: +91-11-42208000, 42208111 Mobile: +91-9810521400
Fax: +91-11-42208444 Email: customerservice@lifetechindia.com Website: www.lifetechindia.com