



Biosystems™

*The Transfection &
Gene Expression Experts*

HL-60 Cell Avalanche® Transfection Reagent

Cat. No. EZT-HL60-1

Size: 0.5 ml
1.5 ml

Store at 4°C

Cell Line Information:

Designations: HL-60

Organism: Homo sapiens (human)

Tissue: peripheral blood; acute promyelocytic leukemia; promyeloblast

Age: 36 years

Gender: female

Ethnicity: Caucasian

Morphology: myeloblastic

Growth properties: suspension

Tumorigenic: yes, form colonies in semi-solid media and produce subcutaneous myeloid tumors in nude mice.

Receptors expressed: complement; Fc

Products: tumor necrosis factor (TNF), also known as tumor necrosis factor alpha (TNF-alpha, TNF alpha), after stimulation with phorbol myristic acid

Depositors: R.C. Gallo

HL-60 is a promyelocytic cell line derived by S.J. Collins, et al. Peripheral blood leukocytes were obtained by leukopheresis from a 36-year-old Caucasian female with acute promyelocytic leukemia. HL-60 cells spontaneously differentiate and differentiation can be stimulated by butyrate, hypoxanthine, phorbol myristic acid (PMA, TPA), dimethylsulfoxide (DMSO, 1% to 1.5%), actinomycin D, and retinoic acid. The cells exhibit phagocytic activity and responsiveness to chemotactic stimuli. The line is positive for myc oncogene expression.

HL-60 is one of the cell lines of the NCI-60 panel which represents different cancer types and has been widely utilized for drug screening and molecular target identification.

The Transfection Reagent:

Transfection of suspension cells, such as HL-60 Cells, has always been challenging, both technically and financially. The most common systems currently used for relatively efficient molecular delivery to these cells are expensive electroporation instruments. However, electroporation usually leads to cell death, primarily when the electrical fields cause

permanent permeabilization of the membrane and the consequent loss of cell homeostasis, in a process known as irreversible electroporation

HL-60 Cell Avalanche® Transfection Reagent (Hereafter “The Reagent”) is a new class of unique chemical formulations specifically formulated and optimized for transfecting HL-60 Cells. The proprietary formulation of lipids and polymers ensures the highest possible transfection efficiencies and viabilities for HL-60 Cells.

Features:

- Unique formulation-maximize transfection performance in HL-60 Cells.
- Extremely gentle to cells
- Deliver single or multiple plasmids
- Simplest and straightforward protocols
- Compatible with serum
- Suitable for Reverse Transfection
- Reproducible: due to highly controlled chemical synthesis of each of the ingredients, the reagent forms uniformly sized complex particles with nucleic acids. With optimized protocol, our reagent will ensure the reproducible highest transfection results.
- Economical: High efficiency means less amount of nucleic acid & reagent is needed
- Developed and manufactured by EZ Biosystems

BEFORE YOU START:

Important Tips for Optimal Transfection

1. Prepare high-quality plasmid DNA at 0.5–5 µg/µl in deionized water or TE buffer. A GFP (green fluorescent protein) plasmid can be used to determine transfection efficiency.
2. Use Opti-MEM® I Reduced Serum Medium (Life Technologies) or regular DMEM without serum to make The Reagent and nucleic acid mix (Only Opti-MEM® I will be mentioned in the remaining parts of the protocol for simplification purpose). Do not use NaCl₂ solution or PBS.
3. Maintain the same seeding conditions between experiments. Use low-passage cells; make sure that cells are healthy and greater than 90% viable before transfection.
4. The Reagent is extremely gentle to cells. However, transfection process will impose stress on cells, no matter what type of transfection methods you use. The trick is to get the balance between transfection efficiency and cell viability. Increasing the number of cells plated per well or decreasing DNA/The Reagent amount will minimize the effect of transfection on cell growth and viability. With careful adjustment, as described in page 3 and 4, this can be achieved while keeping the highest transfection efficiency.
5. Don't use antibiotics in the culture medium during the first 24 hours of transfection.

Protocols

1 DNA Transfection

1.1 Cell Seeding

For optimal DNA transfection conditions, use low-passage cells; make sure that cells are healthy and greater than 90% viable before transfection. Typically, for experiments in 24-well plates, about 2×10^5 cells are seeded per well in 0.5 ml of fresh medium **without antibiotics** prior to transfection. For other culture formats, refer to Table 1.

Table 1. Recommended number of cells to seed the day before transfection

Culture vessel	Number of cells to seed	Surface area per well (cm ²)	Volume of medium per well to seed the cells (ml)
96-well	* 4×10^4 **(7,500-10,000)	0.3	0.1
24-well	* 2×10^5 **(50,000-80,000)	1.9	0.5
12-well	* 4×10^5 **(80,000-150,000)	3.8	1
6-well/35 mm	* 8×10^5 **(150,000-250,000)	9.4	2
60 mm/flask 25 cm ²	* 2×10^6 **(250,000-800,000)	25-28	5
100 mm/flask 75 cm ²	* 6×10^6 **(1×10^6 - 2×10^6)	75-78.5	10
150 mm/flask 175 cm ²	* 1.3×10^7 **(2×10^6 - 5×10^6)	153-175	25

*For HL-60 cells

**For adherent culture cells (see section 1.4)

1.2 DNA Transfection on HL-60 cells

Due to cell culture variations and passage number differences, HL-60 cells from different sources may have different sensitivity to The Reagent. If this is the first time that you are using The Reagent on your HL-60 cells, follow the following procedures and Figure 1 for fine-tuning optimization (**The fine-tuning optimization procedures are extremely important for successful transfection. The amount of The Reagent needed for maximum transfection on HL-60 cells from different sources may differ dramatically**).

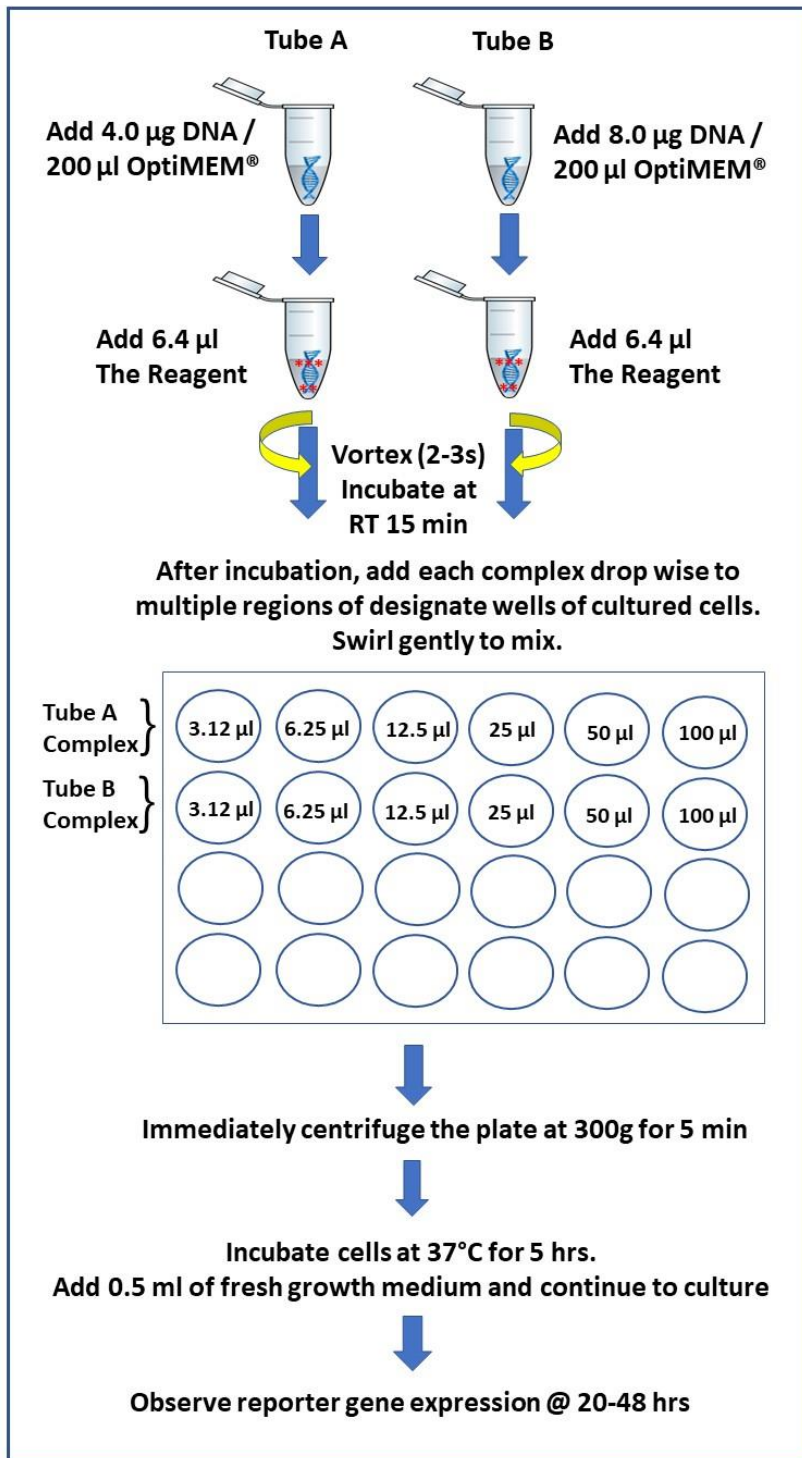
As an example, the following procedures and Figure 1 are for fine-tuning optimization on 24-well plate:

1. Bring The Reagent and serum-free medium (OptiMEM®I) to room temperature before starting.
2. Add 200µl of OptiMEM®I into two 1.5ml tubes (Tube A and B).
3. Add 4.0 µg of DNA to Tube A and 8.0 µg of DNA to Tube B.
4. Mix The Reagent prior to preparing complexes. Add 6.4 µl of The Reagent to both Tube A and Tube B containing 200µl of different concentration of DNA solution (20.0 µg/ml and 40.0 µg/ml respectively). Vortex for 2-3 seconds. Incubate each tube for 15 minutes at room temperature.
5. After incubation, add 3.12, 6.25, 12.5, 25, 50, and 100µl of DNA/The Reagent complexes dropwise directly to the corresponding wells of the 24-well cell culture plate (See Figure 1). Swirl plate gently.
6. Immediately centrifuge the plate at 300 g for 5 min.
7. Incubate the cells at 37°C in a CO2 incubator 5 hours.
8. Add 0.5 ml of fresh growth medium, and continue to culture.

Expression of reporter gene activity should generally be assessed at 20-48 hours post-transfection. GFP expression is maximal at 40-48 hours post-transfection.

After you have completed the fine-tuning optimization steps, choose the amount of DNA/The Reagent that gave you the optimal balance of potency & low cytotoxicity (which usually is the lowest dose that gave you the same high transfection efficiency as other higher doses did) for all of your future experiments on this specific cell type.

Figure 1.



1.3 Scale Up or Down Transfections

Use Table 2 to scale the amount of DNA/The Reagent for your transfection experiment.

Table 2. Scaling Up or Down Transfection Instruction

Culture Vessel	Multiplication factor*
96-well	0.17
48-well	0.50
24-well	1.00
12-well	2.00
6-well	5.00
60-mm	11.05**
10-cm	28.95**
T75	39.47**

**After determining the optimum amount of DNA/The Reagent from the fine-tuning optimization procedures on the above 24-well plate, use the multiplication factor to determine the DNA and The Reagent amount needed for your new plate format.*

***For large format transfections, the centrifugation step may be skipped without obviously affecting transfection efficiency.*

1.4 DNA Transfection on cells other than HL-60 cells

HL-60 Cell Avalanche® Transfection Reagent can also be used on the following cells with high transfection efficiencies.

MDCK Cell, Vero Cell, 293 Cell, 293T/17 Cell, MG-63 Cell, WI-38 Cell, COS-7 Cell

The above protocol usually provides satisfactory transfection efficiency with invisible cytotoxicity on the above cells. However, additional optimization may be needed for certain type of cells. Optimizations may include: cell density; the amount of DNA and The Reagent; DNA/The Reagent ratio, or incubation time for the mixture of The Reagent/DNA etc.

If you want to achieve the best transfection result for a specific type of cells, we recommend using the respective cell type/cell line specific Avalanche® transfection reagents. Those reagents have been optimized on both formulations and protocols, and have been proved to have the best transfection results for the respective cell lines or primary cells. You can easily find the respective Avalanche® Transfection Reagents specific for your cells by using the filters of our product list in EZ Biosystems website:

www.ezbiosystems.com.

2 siRNA Transfection

Though HL-60 Cell Avalanche® Transfection Reagent formulation was optimized for DNA transfection, it can also be used for siRNA transfection. The following are recommended protocol. Protocol optimization may be needed.

2.1 Cell Seeding

For optimal siRNA transfection conditions, use low-passage cells; make sure that cells are healthy and greater than 90% viable before transfection. Typically, for experiments in 6-well plates, about 5×10^5 cells are seeded per well in 2 ml of growth medium without antibiotics 24 h prior to transfection. For other culture formats, refer to Table 3.

Table 3. Recommended number of cells to seed the day before transfection.

Culture vessel	Number of cells to seed	Surface area per well (cm ²)	Medium per well to seed the cells (ml)
24-well	1.25×10^5	1.9	0.5
12-well	2.5×10^5	3.8	1
6-well/35 mm	5×10^5	9.4	2
60 mm/flask 25 cm ²	1.25×10^6	25-28	5
100 mm/flask 75 cm ²	3.75×10^6	75-78.5	10

2.2 siRNA Transfection

We recommend using 10 to 50 nM siRNA (final concentration). The following conditions are given per well of a 6-well plate. For other culture format, please refer to Table 4.

1. Dilute 22 to 110 pmoles siRNA (final concentration: 10 to 50 nM) into 200 µl of Opti-MEM® Reduced-Serum Medium or regular high glucose DMEM without serum. Mix by vortexing.
2. Briefly vortex The Reagent, and add 1.0-5.0 µl into the diluted siRNA. Immediately vortex for 10 s.
3. Incubate for 15 min at RT.
4. Add the transfection mixture drop-wise into each well.
5. Gently rock the plates back and forth and from side to side, and immediately centrifuge the plate at 300 g for 5 min.
6. Gently put in incubator, and incubate at 37 °C CO₂.
7. Analyze after 24 h or later.

Table 4. siRNA transfection guidelines according to the cell culture vessel per well

Culture Vessel	siRNA (pmole) 10 nM	siRNA (pmole) 50 nM	The Reagent (μl)	Opti-MEM or DMEM (μl)	Growth medium (ml)	Final Volume in the well (ml)
24-well	5.5	27.5	*0.2-1.0	50	0.5	0.55
12-well	11	55	*0.4-2.0	100	1	1.1
6-well/ 35 mm	22	110	1.0-5.0	200	2	2.2
60 mm/ flask 25 cm ²	44	220	2.3-11.5	400	4	4.4
100 mm/ flask 75 cm ²	121	605	5.8-29	1100	11	12.1

* Dilute The Reagent 1:5 with H₂O prior application (4 μl reagent + 16 μl H₂O), and then use 5 times of volume for accurate pipetting.

Avalanche® is the trademark of EZ Biosystems LLC. All rights reserved.

Avalanche® series Transfection Reagents are sold to the Buyer with a limited license for Research Use Only and is not for clinical, therapeutic or diagnostic use in humans or animals.

Avalanche® series Transfection Reagents may not be re-packaged or re-sold without written permission from EZ Biosystems LLC. The buyer agrees not to attempt to reverse engineer, reconstruct, synthesize or otherwise modify EZ Biosystems Avalanche® products. A license from EZ Biosystems LLC is required for commercial application of Avalanche® series Transfection Reagents. For obtaining a license to use those products for commercial application, contact EZ Biosystems LLC via business@ezbiosystems.com. For full terms and conditions, visit: www.ezbiosystems.com.