Avalanche®-Everyday Transfection Reagent

Cat. No. EZT-EVDY-1 **Size: 0.75 ml** **Store at 4°C**
1.5 ml

Description

As the simplified version of our most popular and powerful Avalanche®-Omni Transfection Reagent (Cat#: EZT-OMNI-1), Avalanche®-Everyday Transfection Reagent (Avalanche®-Everyday) has the basic formulation of Avalanche®-Omni, which is a new, proprietary formulation of lipids and polymers, thus is also an exceptionally powerful and versatile next-generation DNA and siRNA broad spectrum transfection reagent. Avalanche®-Everyday's transfection efficiency is very close to that of Avalanche®-Omni, but with the prices less than half of Avalanche®-Omni. Best for everyday use on commonly used cells.

Features:

- Broad Spectrum DNA/siRNA delivery - one transfection reagent and protocol for a variety of cells.
- Transfection efficiency is close to that of Avalanche®-Omni Transfection Reagent, but with much lower prices.
- Very low Cellular Toxicity because of its bio-degradability after endocytosis. Maintain cell density, reduce experimental biases.
- Same simple protocol as that of Avalanche®-Omni Transfection Reagent: Does not require removal of serum or culture medium and does not require washing or changing of medium after introducing the reagent/DNA complex.
- Best for everyday use on commonly used cells.
- High levels of recombinant protein production
- Ideal for high-throughput work

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. Make sure the plasmids are endotoxin-free and have A260/280 absorbance ratio of 1.8–2.0. 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 Avalanche®-Everyday and nucleic acid mix (Only Opti-MEM® I will be mentioned in the remaining parts of the protocol for simplification purposes). 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. It is important to have the cells in proliferation state and 70-90% confluence at the time of DNA transfection.
5. Avalanche®-Everyday Transfection 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 and Avalanche®-Everyday amount will minimize the effect of transfection on cell growth and viability. With careful optimization, as described in page 3 and 4, this can be achieved while keeping the highest transfection efficiency.
6. 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, we recommend using cells which are 70% to 90% confluent at the time of transfection. Typically, for experiments in 24-well plates, 50,000-80,000 adherent cells are seeded per well in 0.5 ml of cell growth medium **without antibiotics** 24 h prior to transfection. For the different culture formats, refer to Table 1.

Table 1. Recommended number of cells to seed the day before transfection in culture medium without antibiotics

Culture vessel	Number of Adherent cells to seed (Suspension Cells)	Surface area per well (cm^2)	Volume of medium per well to seed the cells (ml)
96-well	7,500-10,000 ($4x10^4$)	0.3	0.1
24-well	50,000-80,000 ($2x10^5$)	1.9	0.5
12-well	80,000-150,000 ($4x10^5$)	3.8	1
6-well/35 mm	150,000-250,000 ($8x10^{5)}$	9.4	2
60 mm/flask 25 cm^2	250,000-800,000 ($2x10^6$)	25-28	5
100 mm/flask 75 cm^2	$1x10^6$-$2x10^6$ ($6x10^6$)	75-78.5	10
150 mm/flask 175 cm^2	$2x10^6$-$5x10^6$ ($1.3x10^7$)	153-175	25

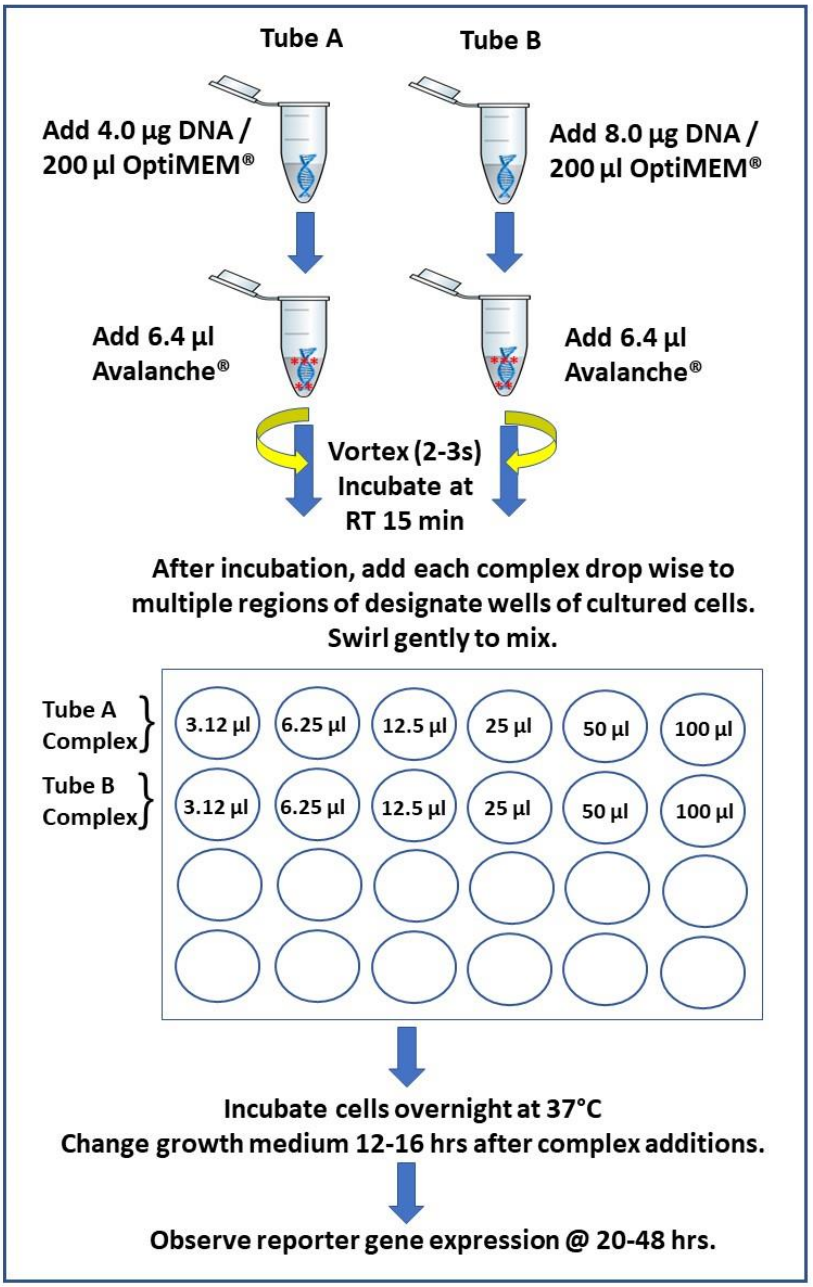
1.2 DNA Transfection

If this is the first time that you are using Avalanche®-Everyday on a specific type of cells, follow the following procedures and Figure 1 for optimization **(The optimization procedures are extremely important for successful transfection. Since different types of cells have different sensitivity to Avalanche®-Everyday, the amount of Avalanche®-Everyday as well as the amount of DNA needed for maximum transfection on different types of cells may differ dramatically).**

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

1. Bring Avalanche®-Everyday 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 Avalanche®-Everyday prior to preparing complexes. Add 6.4 µl of Avalanche®- Everyday 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/Avalanche®- Everyday complexes dropwise directly to the corresponding wells of the 24-well cell culture plate (See Figure 1). Swirl plate gently.
6. Incubate the cells at 37°C in a CO2 incubator
7. Change growth medium 12-16 hours later.
8. 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.

Figure 1.



After you have completed the optimization steps, choose the amount of DNA and Avalanche®-Omni 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.

1.3 Scale Up or Down Transfections

Use Table 2 to scale the amount of DNA/Avalanche®-Everyday for your transfection experiment.

Table 2. Scaling Up or Down Transfection Instruction

Culture Vessel	Multiplication factor[1]
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

[1]After determining the optimum amount of DNA/Avalanche®-Everyday from the optimization procedures on the above 24-well plate, use the multiplication factor to determine the DNA and Avalanche®-Everyday amount needed for your new plate format.

2 siRNA Transfection

2.1 Cell Seeding

For optimal siRNA transfection conditions, we recommend using cells which are 50% confluent at the time of transfection. Typically, for experiments in 6-well plates, 100,000 to 150,000 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 in culture medium without antibiotics

Culture vessel	Number of adherent cells to seed	Surface area per well (cm^2)	Medium per well to seed the cells (ml)
24-well	25,000-40,000	1.9	0.5
12-well	50,000-80,000	3.8	1
6-well/35 mm	100,000-150,000	9.4	2
60 mm/flask 25 cm^2	200,000-500,000	25-28	5
100 mm/flask 75 cm^2	$0.5x10^6$-$1x10^6$	75-78.5	10

2.2 siRNA Transfection

For optimal siRNA-mediated silencing, 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 formats, 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 Avalanche®-Everyday, 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 return the plate to the 37°C CO2 incubator.
6. Analyze after incubating for 24 h or longer.

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

Culture Vessel	siRNA (pmole) 10 nM	siRNA (pmole) 50 nM	Avalanche®-Everyday (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 Avalanche®-Everyday 1:5 with H_2O prior to application (4 µl reagent + 16 µl H_2O), and then use 5 times of the volume in the table for accurate pipetting.

Intended Use:

All Avalanche® Series Transfection Reagents are for research use only, not intended for any animal or human therapeutic or diagnostic use.

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