Avalanche®-Omni Transfection Reagent

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

Description

Avalanche®-Omni Transfection Reagent is a proprietary lipid-polymer mixture that forms lipopolyplexes with nucleic acids. It is an exceptionally powerful and versatile next-generation DNA and siRNA transfection reagent for day-to-day experiments. It has been shown to effectively transfect the broadest spectrum of adherent and suspension cells. No other transfection reagents can match the efficiency, broad spectrum capabilities, convenience, and gentleness of Avalanche®-Omni Transfection Reagent for transfection of primary cells and other hard-to-transfect cells. Because of the high transfection efficiency, much lesser amounts of DNA and Avalanche®-Omni are needed to achieve high transfection efficiency as compared to most other transfection reagents. It is best to use the smallest amounts of DNA and transfection reagent possible, in order to maintain good cell growth and viability, and generate physiologically relevant data you can trust.

Features:

- Exceptional transfection efficiency in the broadest range of cell types including difficult-to-transfect cell lines and primary cells with super high levels of gene expression or knockdown.
- Economical: High efficiency means smaller amounts of the nucleic acid and reagent are needed. 1.5 ml of Avalanche®-Omni Reagent is sufficient for about 3,500 transfections in 24-well plates, or about 700 transfections using 6-well plates!
- Extremely gentle to cells: The chemical features of our proprietary formula and the smaller amounts needed for transfection ensure very low toxicity levels.
- Simplest procedure: – simply mix with nucleic acid and add to cell culture.
- Superior performance for co-transfection of siRNA and plasmid DNA
- Proven efficacy in the presence of serum – eliminates the need to change media following transfection
- 100% animal origin- free
- Reliable performance for high-throughput applications
- The best choice for establishing stable cell lines

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®-Omni Transfection Reagent and nucleic acid mix. Do not use $NaCl_2$ 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®-Omni 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 the Avalanche®-Omni 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 6-well plates, 150,000-250,000 adherent cells are seeded per well in 2 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®-Omni on a specific type of cells, first, transfect the cells according to Table 2a for optimization **(The optimization procedures are extremely important for successful transfection. Since different types of cells have different sensitivity to Avalanche®-Omni, the amount of Avalanche®-Omni needed for maximum transfection on different types of cells may differ dramatically).**

Table 2a. Transfection optimization guidelines according to the cell culture vessel per well

Component	**96-well**	**24-well**	**6-well**
Opti-MEM Medium (µl)	250	250	1200
DNA (µg)	2.5	2.5	12
Diluted DNA (µl)	4 x 50 µl	4 x 50 µl	4 x 250 µl
Avalanche®-Omni (µl)	*0.2, *0.4, *0.7, 1.0	*0.2, *0.4, *0.7, 1.0	1.0, 2.0, 3.5, 5.0
Incubate for 15 minutes at room temperature			
DNA-reagent complex/well (µl)	**10**	**50**	**250**
Incubate cells for 24 hours or more at 37°C before analysis			

*Dilute Avalanche®-Omni 1:5 with H_2O prior to application (4 µl reagent + 16 µl H_2O), and then use 5 times of the volumes in the table for accurate pipetting.

Table 2b shows the amounts of DNA and Avalanche®-Omni per well used in each of the above transfection reactions.

Table 2b. Amount of DNA and Avalanche®-Omni per well

Amount	96-well	24-well	6-well
DNA/well (ng)	100	500	2500
Avalanche®-Omni (µl)	0.04-0.2	0.2-1.0	1.0-5.0

As an example, the following steps are given for optimization on 6-well plate. For other culture formats, please refer to Table 2 and Table 3.

1. Transfer 12.0 µg DNA into 1200 µl Opti-MEM® Reduced-Serum Medium (Cat# 31985-070, Life Technologies) or regular high glucose DMEM without serum. Mix by vortexing. Aliquot 4 x 250 µl of the above DNA solution into 4 x 1.5 ml Eppendorf tubes.
2. Briefly vortex Avalanche®-Omni, and add 1.0, 2.0, 3.5, and 5.0 µl into the above diluted DNA respectively. Immediately vortex for 5 s after each addition.
3. Incubate for 15 min at RT.
4. Add the 250 µl transfection mixture drop-wise into each well (Note: for the 96-well format, the amount of transfection mixture added per well is only part of the total volume as indicated in Table 2).
5. Gently rock the plates back and forth and from side to side, and return the plate to the 37 °C CO2 incubator. It is not necessary to remove complexes or to change/add medium after transfection.
6. Analyze after incubating for 24 h or longer.
7. For stable transfection, start the antibiotic selection 24 – 48 h after transfection. Grow cells in selective medium for 10-15 days.

After you have completed the optimization steps, choose the amount of Avalanche®-Omni that gave you the optimal balance of potency & low cytotoxicity for all of your future experiments on this specific cell type.

1.3 Scale Up or Down Transfections

Use Table 3 to scale the volumes for your transfection experiment.

Table 3. Scaling Up or Down Transfection Instruction

Culture Vessel	Multiplication factor[1]	Vol. Complex-Opti-MEM per well (µl)	DNA (µg)	Avalanche®-Omni (µl)
96-well	0.17	10	0.1	0.04-0.2
48-well	0.50	25	0.25	0.1-0.5
24-well	**1.00**	**50**	0.5	**0.2-1.0**
12-well	2.00	100	1.0	0.4-2.0
6-well	5.00	200	2.5	1.0-5.0
60-mm	11.05	500	5.5	2.3-11.5
10-cm	28.95	1000	14	5.8-29
T75	39.47	1500	20	7.9-39

[1]After determining the optimum reagent amount, use the multiplication factor to determine the reagent amount needed for your new plate format.

[2]Optimum amount needed is determined from the protocol in the previous two pages.

1.4 Virus Production

Avalanche®-Omni is ideal for virus production, especially retrovirus, AAV and lentivirus, in adherent cells (ex: HEK-293T). For co-transfection of multiple plasmids, the total DNA amount per well/plate should not exceed the DNA amount indicated in Table 3. The ratio to use for each plasmid depends on the size of the plasmids, the plasmid constructs and the desired expression level of each plasmid. Please adjust the ratios according to your application. Each plasmid should represent at least 10% of the total DNA amount per well/plate. The following conditions are given per 100 mm dish for HEK-293T cells. If using other types of cells or other formats, optimize and scale according to Table 2 and Table 3.

1. Dilute 7 µg total DNA amount into 1000 µl Opti-MEM or DMEM. Mix by vortexing.
2. Add 7-10 µl (may need adjustment for maximum yields) Avalanche®-Omni, vortex for 10 s.
3. Incubate for 15 min at RT.
4. Add the transfection mix per dish drop wise onto the cells in the serum containing medium, and distribute evenly.
5. Gently rock the dish back and forth and from side to side.
6. Incubate in tissue culture incubator for 4h. Replace transfection medium with cell growth medium and return the dish to the incubator.
7. Incubate 24 to 72 h and proceed to virus purification and titration.

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 4.

Table 4. 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 5.

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®-Omni, 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 5. siRNA transfection guidelines according to the cell culture vessel per well

Culture Vessel	siRNA (pmole) 10 nM	siRNA (pmole) 50 nM	Avalanche®-Omni (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®-Omni 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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