Biosystems™ *The Transfection & Gene Expression Experts*

Avalanche®-CRISPR Transfection Reagent

Cat. No. EZT-CRPR-1 **Size: 0.5 ml** **Store at 4°C**
1.5 ml

CRISPR-Cas9–mediated genome targeting:

CRISPR-Cas9 has triggered a revolution in which laboratories around the world are using the technology for innovative applications in biology. This technology is able to systematically analyze gene functions in mammalian cells, study genomic rearrangements and the progression of cancers or other diseases, and potentially correct genetic mutations responsible for inherited disorders.

The CRISPR-Cas system is a prokaryotic immune system that confers resistance to foreign genetic elements such as plasmids and phages, and provides a form of acquired immunity. CRISPR spacers recognize and cut these exogenous genetic elements in a manner analogous to RNA interference in eukaryotic organisms. CRISPRs are found in approximately 40% of sequenced bacteria genomes and 90% of sequenced archaea.

The CRISPR interference technique can be applied in eukaryotic cells. By delivering the Cas9 protein and appropriate guide RNAs (sgRNA) into a cell, the organism's genome can be cut or modified at any desired locations.

The Transfection Reagent:

One of the most important factors that determines a successful CRISPR-Cas9–mediated genome editing is the efficiency of delivering functional Cas9 gene and sgRNA into the cells (the transfection process). EZ Biosystems, the transfection and gene expression expert, has specifically designed, and successfully developed a transfection reagent for CRISPR-Cas9 gene editing in mammalian cells ---- Avalanche®-CRISPR Transfection Reagent.

Features:

- Specifically designed for CRISPR-Cas9 gene editing transfection in mammalian cells
- Maximum transfection efficiency for transfecting large plasmids (>9.0 kb)
- Great for co-transfection of large plasmids with either small plasmids or small RNA, such as sgRNA
- Great for CRISPR-Cas9 gene editing in difficult-to-transfect cells, such as primary cells, suspension culture cells, or neural cells.
- Lowest Cellular Toxicity-maintain cell density and reduce experimental biases
- Compatible with serum
- Chemically defined compounds and completely free of animal-derived components.

BEFORE YOU START:

Important Tips for Optimal Transfection

- Prepare high-quality pCas9 expression plasmids and sgRNA expression plasmids at 1.0–5 µg/µl in deionized water or TE buffer.
- Use Opti-MEM® I Reduced Serum Medium (Life Technologies) or regular DMEM without serum to make Avalanche®-Omni and nucleic acid mix (Only Opti-MEM® I will be mentioned in the remaining parts of the protocol for simplification purposes). Do not use $NaCl_2$ solution or PBS.
- Maintain the same seeding conditions between experiments. Use low-passage cells; make sure that cells are healthy and greater than 90% viable before transfection.
- It is important to have the cells in proliferation state and 70-90% confluence at the time of DNA transfection.
- Avalanche®-CRISPR 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 Avalanche®-CRISPR Transfection Reagent/DNA amount will minimize the effect of transfection on cell growth and viability. With careful optimization, as described in page 4 and 5, this can be achieved while keeping the highest transfection efficiency.
- Don’t use antibiotics in the culture medium during the first 24 hours of transfection.

Protocols

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 other 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)
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

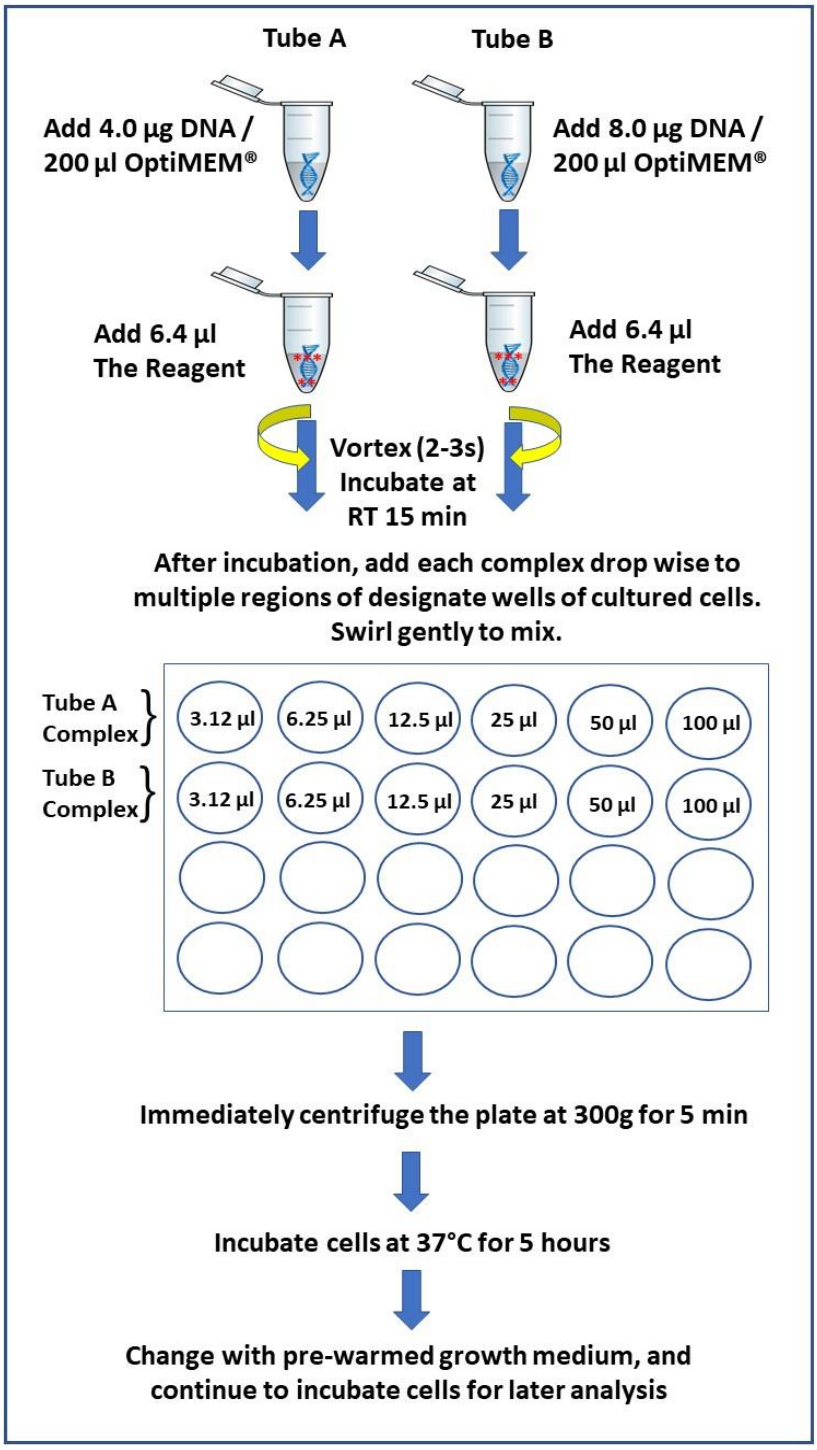
2. Transfection

- Change with fresh media for the cells before transfection.
- Mix pCas9 expression plasmid and sgRNA expression plasmids at the ratios of 1:1 ~ 1:4 (w/w) or 1:2 ~ 1:10 (molar ratio). The optimal ratios vary based on the properties of different Cas9 expression plasmids and sgRNA expression plasmids, and should be empirically determined.
- If this is the first time that you are using Avalanche®-CRISPR Transfection Reagent 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®-CRISPR Transfection Reagent, the amount of Avalanche®-CRISPR Transfection Reagent needed for maximum transfection on different types of cells 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) Change growth medium, and continue to culture in the incubator.
9) Cells were incubated at 37ºC for 48 hours or more post transfection before genomic DNA extraction for analysis or cell selection/isolation.

Figure 1.



After you have completed the fine-tuning optimization steps, choose the amount of DNA and 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.

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.*

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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