Biosystems™ *The Transfection & Gene Expression Experts*

Avalanche®-CRISPR Transfection Reagent

Cat. No. EZT-CRSP-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 experts, 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 The Reagent and nucleic acid mix. 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.
- 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 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.
- 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 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)
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, 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®-CRISPR Transfection Reagent, the amount of Avalanche®-CRISPR Transfection Reagent 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	24-well	6-well
Opti-MEM Medium (µl)	250	1200
Total plasmid DNA (µg)	2.5	12
Diluted plasmid DNA (µl)	4 x 50 µl	4 x 250 µl
Avalanche®-CRISPR Transfection Reagent (µl)	*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)	**50**	**250**
Immediately centrifuge the plate at 300 g for 5 min		
Gently put in incubator, and Incubate cells for 48 hours or more at 37°C		

*Dilute Avalanche®-CRISPR Transfection Reagent 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 total DNA and Avalanche®-CRISPR per well used in each of the above transfection reactions.

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

Amount	24-well	6-well
Total DNA/well (ng)	500	2500
Avalanche®-CRISPR (µl)	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 total 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®-CRISPR Transfection Reagent, 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.
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 CO_2. It is not necessary to remove complexes or to change/add medium after transfection.
7) Cells were incubated at 37ºC for 48 hours or more post transfection before genomic DNA extraction for analysis or cell selection/isolation

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

3. Scale Up or Down Transfections

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

Table 3. Scaling Up Transfection Instruction

Culture Vessel	Multiplication factor[1]	Vol. Complex-Opti-MEM per well (µl)	Total DNA (µg)	Avalanche®-CRISPR (µl)
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.

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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