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 is a genome-editing technology adapted from a natural defense system in bacteria and archaea. In mammalian cells, a single-guide RNA (sgRNA) directs the Cas9 enzyme to a matching DNA sequence next to a compatible PAM sequence. Cas9 cuts the DNA, and the cell's repair processes can then disrupt or alter the targeted gene.

Researchers use CRISPR-Cas9 to investigate gene function, model genomic changes involved in cancer and other diseases, and explore ways to correct disease-causing mutations. Successful editing depends on delivering the CRISPR components into the target cells and verifying the resulting DNA changes.

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 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 (The Reagent, hereafter) with the maximum possible transfection efficiency.

Features:

- Specifically designed for CRISPR-Cas9 gene editing transfection in mammalian cells
- Supports multiple CRISPR-Cas9 delivery formats: plasmid(s) encoding Cas9 and gRNA; a Cas9-encoding plasmid co-delivered with gRNA; Cas9 mRNA co-delivered with gRNA; or a preassembled Cas9–gRNA ribonucleoprotein (RNP) complex.
- Compatible with TrueGuide™ Synthetic gRNA, TrueCut™ Cas9 Protein v2, and CRISPR libraries from Thermo Fisher Scientific.
- 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

- Use low-passage cells; make sure that cells are healthy and greater than 90% viable before transfection.
- Cell density at the time of transfection is critical. Use cells between 40–70% confluent at time of transfection. Test different cell seeding densities to determine the optimal confluence for transfection. Maintain the same seeding conditions between experiments.
- Cell seeding number is based on growth rate. Seed fewer cells for fast growing cells.
- 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. With careful optimization, as described in page 4, 5, and 6, 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 30% to 70% confluent at the time of transfection. Typically, for experiments in 24-well plates, 25,000-40,000 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	25,000-40,000 ($1x10^5$)	1.9	0.5
12-well	40,000-75,000 ($2x10^5$)	3.8	1
6-well/35 mm	75,000-125,000 ($4x10^{5)}$	9.4	2
60 mm/flask 25 cm^2	125,000-400,000 ($1x10^6$)	25-28	5
100 mm/flask 75 cm^2	$5x10^5$-$1x10^6$ ($3x10^6$)	75-78.5	10
150 mm/flask 175 cm^2	$1x10^6$-$2.5x10^6$ ($6.5x10^6$)	153-175	25

2. Transfection

2.1. CRISPER gene editing with Cas9 and gRNA plasmid(s):

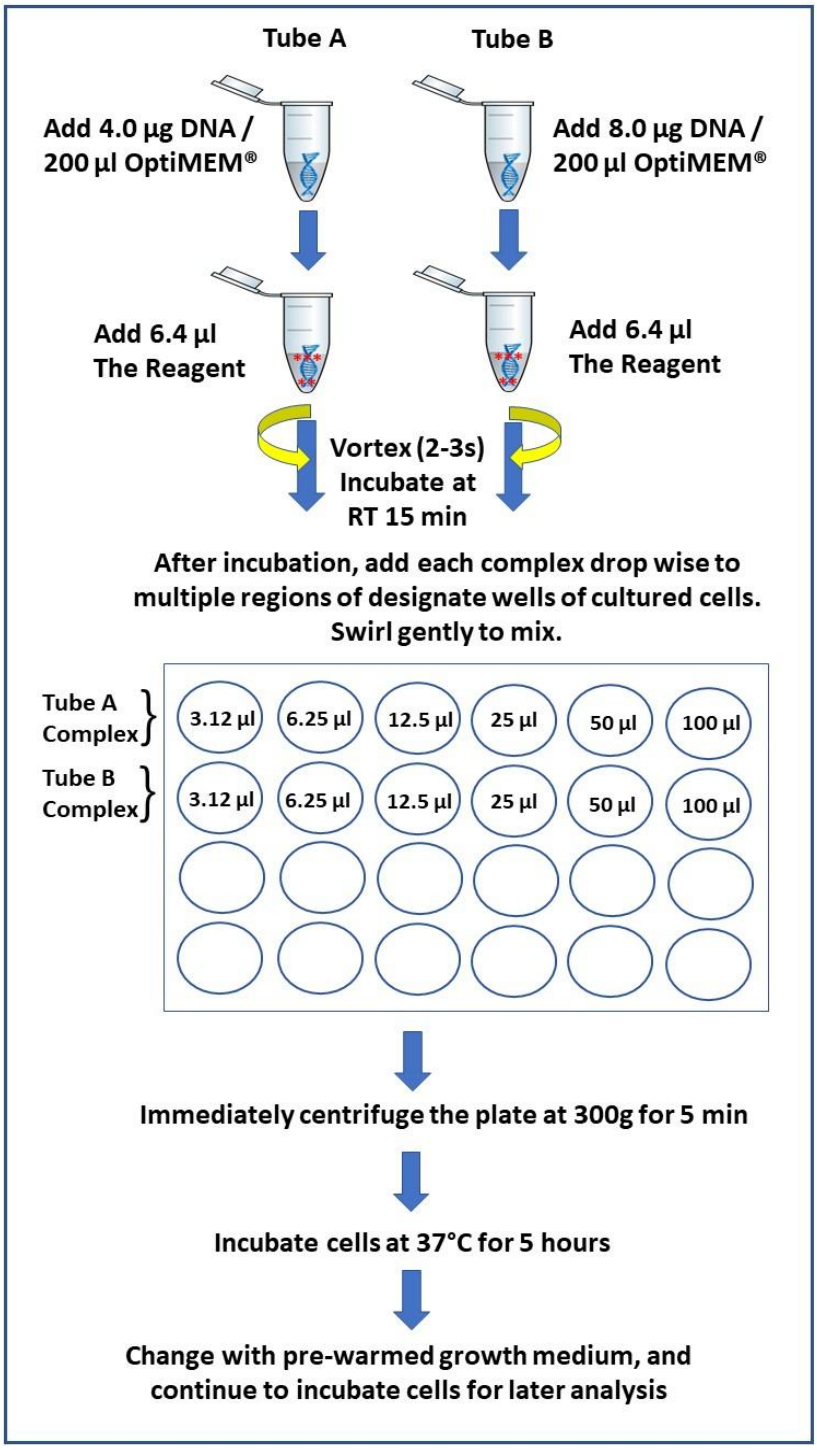
Use highly purified, sterile, and contaminant-free DNA for transfection. Plasmid DNA preparations that are endotoxin-free and have A260/280 absorbance ratio of 1.8–2.0 are desirable.

- 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. **mRNA transfection uses the same condition as plasmid transfection**.
- 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 optimization on 24-well plate:

1) Bring The Reagent and serum-free medium (Opti-MEM® I) to room temperature before use.
2) Add 200 µl of Opti-MEM® I to each of two 1.5-ml tubes labeled 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 before use. Add 6.4 µl to each tube, vortex for 2–3 seconds, and incubate for 15 minutes at room temperature to allow complexes to form.
5) Add 3.12, 6.25, 12.5, 25, 50, or 100 µl of the DNA/The Reagent complexes dropwise to the corresponding wells of a 24-well cell culture plate (see Figure 1). Gently swirl the plate to mix.
6) Optional: Immediately centrifuge the plate at 300 × g for 5 minutes.
7) Incubate the cells at 37°C in a CO_2 incubator for 5 hours.
8) Replace the medium with fresh growth medium and continue incubation.
9) At 48 hours or later after transfection, harvest the cells for genomic DNA extraction and analysis, or proceed with cell selection or isolation.
10) After optimization, select the DNA and The Reagent amounts that provide the best balance between transfection efficiency and low cytotoxicity. This is usually the lowest dose that achieves transfection efficiency comparable to higher doses. Use these conditions for subsequent experiments with this cell type.

Figure 1.



2.2. **CRISPER gene editing with Cas9 nuclease/gRNA complex**

Transfect cells according to the following tables. Reaction mix volumes are for one well and account for pipetting variations. Choose one of the following formats. Scale the volumes proportionally for additional wells.

Table 2a. Optimization for Cas9 nuclease transfection with synthetic gRNA according to the cell culture vessel

Component	96-well	24-well	6-well
Opti-MEM Medium (µl)	250	250	1250
Cas9 nuclease (ng)	6250	6250	31250
Synthetic gRNA (ng)	1200	1200	6000
Diluted Cas9 + gRNA (µl)	4 x 50 µl	4 x 50 µl	4 x 250 µl
Avalanche®-CRISPR (µl)	*0.4 *0.7, 1.0, 1.5	*0.4 *0.7, 1.0, 1.5	2.0, 3.5, 5.0, 7.5
Incubate for 10-15 minutes at room temperature			
Complex solution added to cell (µl)	**10**	**50**	**250**
Incubate cells for 2-3 days at 37°. After incubation, remove culture medium and rinse cells with 50–500 µL PBS, lyse with 20–250 µL lysis buffer, and perform genomic cleavage detection assay			

*Dilute Avalanche®-CRISPR 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 *Amount of Cas9 nuclease, gRNA (synthetic), and Avalanche®-CRISPR per well* used in each of the above transfection reaction formats.

Table 2b. Amount of Cas9 nuclease, gRNA (synthetic), and Avalanche®-CRISPR per well

Amount	96-well	24-well	6-well
Cas9 nuclease/well (ng)	250	1250	6250
gRNA (synthetic) (ng)	48	240	1200
Avalanche®-CRISPR (µl)	0.08-0.3	0.4-1.5	2.0-7.5

Table 3a. Optimization for Cas9 nuclease transfection with in vitro transcribed gRNA according to the cell culture vessel

Component	96-well	24-well	6-well
Opti-MEM Medium (µl)	250	250	1250
Cas9 nuclease (ng)	6250	3125	31250
gRNA (IVT) (ng)	1200	625	6000
Diluted Cas9 + gRNA (µl)	4 x 50 µl	4 x 50 µl	4 x 250 µl
Avalanche®-CRISPR (µl)	*0.4 *0.7, 1.0, 1.5	*0.4 *0.7, 1.0, 1.5	2.0, 3.5, 5.0, 7.5
Incubate for 10-15 minutes at room temperature			
Complex solution added to cell (µl)	**10**	**50**	**250**
Incubate cells for 2-3 days at 37°. After incubation, remove culture medium and rinse cells with 50–500 µL PBS, lyse with 20–250 µL lysis buffer, and perform genomic cleavage detection assay			

*Dilute Avalanche®-CRISPR 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 3b shows the *Amount of Cas9 nuclease, gRNA (in vitro transcribed), and Avalanche®-CRISPR per well* used in each of the above transfection reaction formats.

Table 3b. Amount of Cas9 nuclease, gRNA ((in vitro transcribed), and Avalanche®-CRISPR per well

Amount	96-well	24-well	6-well
Cas9 nuclease/well (ng)	125	625	3125
gRNA (IVT) (ng)	25	125	625
Avalanche®-CRISPR (µl)	0.08-0.3	0.4-1.5	2.0-7.5

As an example, the following steps are given for optimization of Cas9 nuclease transfection with synthetic gRNA on 24-well plate. For other culture formats, please refer to Table 2, 3, and Table 4.

1) Transfer 6.25 µg Cas9 nuclease and 1.2 ug of synthetic gRNA into 250 µl Opti-MEM® Reduced-Serum Medium (Cat# 31985-070, Life Technologies) or regular high glucose DMEM without serum. Mix by vortexing. Aliquot 4 x 50 µl of the above solution into 4 x 1.5 ml Eppendorf tubes.
2) Briefly vortex Avalanche®-CRISPR, and add 0.4, 0.7, 1.0, and 1.5 µl into the above diluted solution respectively. Immediately vortex for 5 s after each addition.
3) Incubate for 10-15 min at RT.

4) Add the 50 µl 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) Change medium 5 hours after transfection.
7) Incubate cells for 2–3 days at 37°C. After incubation, remove culture medium and rinse cells with 50–500 µl PBS, lyse with 20–250 µL lysis buffer, and perform genomic cleavage detection assay.

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

2.3. **Scale Up or Down Transfections**

Use Table 4 to scale up or down for your transfection experiment.

Table 4. 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 the optimization procedures, use the multiplication factor for other culture vessel transfections.*

********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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