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EZ Transfer

Product Number: TR9810

Shipping and Storage

This product is transported at low temperature and stored at -20℃, with a validity period of 1 year to avoid repeated freezing and thawing. If it cannot be used all at once, 0.1ml can be divided into small portions for 0.2cm electric rotary cups, and 0.25ml can be divided into small portions for 0.4cm electric rotary cups.

Description

EZ Transfer electrotransfection reagent (electrotransfection buffer) is used to efficiently and low toxicity transfect nucleic acids (DNA, siRNA, etc.) into difficult to transfect cells. EZ Transfer is compatible with various conventional electrometers, including Lonza Amaxa®Nucleofector®,Bio-Rad®Gene Pulser and Harvard-BTX® electroporators. EZ Transfer can be used for exponential decay and square wave form electroporation experiments.

Important optimization tips

1. **Lonza (Amaxa) cell nuclear power transfer instrument**

   When using the EZ Transfer transfection reagent with the Lonza (Amaxa) cell nuclear transfection instrument, please follow the operating guidelines and optimization plan of the Lonza (Amaxa) cell nuclear transfection instrument, just replace the electroporation mixture with EZ Transfer.

2. **Cell density during electroporation**

   Determine the optimal cell density for each cell type to maximize the efficiency of electroporation. In the final system containing electroporation reagents, **the cell density during electroporation is generally within the range of 1-10×10^6 cells/ml. For suspended cells, it is best to have a high cell density close to 10×10^6 cells/ml. For adherent cells, the recommended cell density is 1-5×10^6 cells/ml.** You can refer to Tables 1 and 2 to understand the cell density at which EZ Transfer reagents start.

3. **Nucleic acid purity and concentration**

   3.1. **Do not use DNA purified solely by ethanol precipitation method.** The residual salt in the ethanol precipitation method can cause a change in current and have a negative impact on electroporation.

   The recommended concentration range for DNA extraction is 1-5 mg/ml, dissolved in pure water or TE. The use of high concentration DNA may lead to uneven mixing with cells. A lower nucleic acid concentration may dilute the electroporation mixture.

   3.2. **SiRNA uses high-purity and sterile siRNA.**Determine the optimal siRNA concentration for electrotransfection. It is recommended to conduct gradient experiments with different concentrations of siRNA within the final concentration range of 250-750nM.

4. **Pulse condition optimization**

   The EZ Transfer electrotransfection reagent can be used for both exponential decay electroporation experiments and square wave electroporation experiments. Some types of cells are better transfected in the form of square wave pulses, while others respond better to exponential decay forms.

5. **Cell incubation time after electrotransfection**

   There is an optimal incubation time for each cell type after electrotransfection. For plasmid electrotransfection, the optimal incubation time is generally 4-48 hours, and the specific optimal time needs to be adjusted according to the experimental purpose, plasmid properties, and half-life of the expressed protein.

6. **The issue of cell death in electrotransfection**

   When using EZ Transfer, due to the damage of electroporation to cells, according to experience, the transfection effect is best when the mortality rate after transfection is around 50%.

Experimental plan for electroporation

For the Lonza (Amaxa) nuclear transfection machine, it is recommended to follow the Lonza (Amaxa) operation optimization guidelines and simply replace the electroporation mixture with EZ Transfer. The following steps involve conducting electroporation experiments on plasmid DNA, siRNA, or other nucleic acids using other electrotransfection instruments capable of generating exponential decay or square wave pulses in a 0.2cm or 0.4cm electroporation cup.

1. Prepare cells

Cell passage should be conducted 1-3 days before electroporation to maintain exponential growth during the electroporation experiment and achieve optimal cell fusion.

For adherent cells: the cell fusion degree is 70-80%.

For suspended cells: the cell density is 1-2×10^6 cells/ml.

2. Program settings for electric rotary instruments

The exponential decay electroporation instrument has some reference conditions for cell electroporation as shown in Table 1

The reference conditions for partial cell electroporation in the square wave pulse electroporation instrument are shown in Table 2

The specific optimization parameter methods are as follows:

2.1. Exponential decay

When using a 0.4cm electric rotary cup, the exponential decay pulse conditions of most cell types are within the voltage range of 200-300V and the capacitance range of 800-1000μF. For a 0.2 cm electric rotary cup, the range is 80-160V and 800-1000μF, respectively. For the electroporation experiment using a 0.4 cm electric rotary cup, the test voltage range is 200-300V and the capacitance range is 800-1000μF. Firstly, maintain the voltage constant at 220V and change the capacitance in increments of 100μF starting from 750μF. Subsequently, starting from 200V, change the voltage in a gradient of 10V while maintaining a constant capacitance.

2.2. Square wave

Generally speaking, the theoretical starting condition of square wave pulses can be determined by the exponential decay parameter, that is, reducing the pulse length by half and increasing the voltage by about 10% while keeping the capacitance constant. The subsequent optimization can increase or decrease the pulse voltage near the theoretical calculation with a gradient of 10V. The recommended square wave pulse conditions are shown in Table 2. If the exponential decay pulse condition is not known, the optimal square wave pulse condition can be found by testing the voltage, capacitance, and pulse length range. When using a 0.4cm electric rotary cup, the general square wave pulse conditions for most cell types are within the voltage range of 200-300V and the capacitance range of 800-1000μF. For a 0.2 cm electric rotary cup, the range is 80-160V and 800-1000μF, respectively. The pulse length ranges from 10 to 20 milliseconds.

In addition to voltage and capacitance settings, some electroporation testers also provide settings for resistance (ohms, Ω). When using EZ Transfer for electro-hydraulic conversion, the ideal resistance is none, zero ohms (Ω), or ∞. If your electroporation instrument has resistance options other than zero ohms, please use the lowest possible resistance.

Table 1. Recommended program settings for electroporation using EZ Transfer electroporation fluid and exponential decay pulse electroporation instrument

Cell Type	Cuvette Size (cm)	Cell Density (×10^6)cells/ml	DNA (μg)	Electroporation Volume (μl)	Voltage (V)	Capacitance (μF)
Primary Human Keratinocyte	0.2	2	2	100	150	950
	0.4		5	250	220	950
Primary MEFs	0.2	5	2	100	150	950
	0.4		5	250	230	950
Primary Rat Cortical Neuron	0.2	1	2	100	120	950
	0.4		-	-	-	-
A-549	0.2	5	-	-	-	-

	0.4		5	250	280	950
BHK-21	0.2	10	2	100	150	950
	0.4		5	250	280	950
CHO-K1	0.2	5	2	100	150	950
	0.4		5	250	280	950
COS-7	0.2	5	2	100	150	950
	0.4		5	250	260	950
HEK-293	0.2	5	2	100	160	950
	0.4		5	250	250	950
HEK-293T	0.2	5	-	-	-	-
	0.4		5	250	250	950
HeLa	0.2	3	2	100	130	950
	0.4		5	250	260	950
Hepa	0.2	5	2	100	160	950
	0.4		-	-	-	-
HepG2	0.2	5	2	100	170	950
	0.4		5	250	250	950
HL-60	0.2	10	2	100	150	950
	0.4		5	250	275	950
HUV-EC	0.2	3	-	-	-	-
	0.4		5	250	250	950
Jurkat E6-1	0.2	10	2	100	150	950
	0.4		5	250	260	950
K562	0.2	10	2	100	130	950
	0.4		5	250	250	950
MCF-7	0.2	3	2	100	150	950
	0.4		-	-	-	-
NIH-3T3	0.2	10	2	100	160	950
	0.4		5	250	260	950
NIKS	0.2	2	2	100	170	950
	0.4		5	250	280	950
PC-12	0.2	3	2	100	130	950
	0.4		5	250	240	950
RAW 264.7	0.2	5	2	100	150	950
	0.4		5	250	260	950
SH-SY5Y	0.2	5	-	-	-	-
	0.4		5	250	250	950
SK-BR-3	0.2	5	2	100	160	950
	0.4		5	250	260	950
SK-N-MC	0.2	5	2	100	90	950
	0.4		5	250	240	950
THP-1	0.2	10	2	100	140	950
	0.4		5	250	250	950
U-937	0.2	10	-	-	-	-

	0.4		5	250	260	950
Vero	0.2	5	2	100	170	950
	0.4		-	-	-	-
Other cell types	0.2	5-10	2	100	80-160	800-1000
	0.4		5	250	200-300	800-1000

Table 2. Recommended program settings for electroporation using EZ Transfer electroporation fluid and square wave pulse electroporation instrument

Cell Type	Cuvett e Size(cm)	Cell Densit y($\times10^6$) cells/ml	DNA(μg)	Electroporatio n Volume(μl)	Voltage(V)	Capacitanc e(μF)	Pulse Lengt h(mSec)
Primary Human Keratinocyte	0.2	2	2	100	170	950	10
	0.4		5	250	250	950	15
Primary MEFs	0.2	5	2	100	170	950	10
	0.4		5	250	280	950	15
A-549	0.2	5	-	-	-	-	-
	0.4		5	250	280	950	15
Jurkat E6-1	0.2	10	2	100	180	950	10
	0.4		5	250	275	950	15
NIH-3T3	0.2	10	2	100	160	950	10
	0.4		5	250	260	950	15
NIKS	0.2	2	2	100	180	950	10
	0.4		-	-	-	-	-
Other cell types	0.2	5-10	2	100	80-160	800-1000	10-20
	0.4		5	250	200-300	800-1000	10-20

3. Electric perforation experiment

3.1. Calculate the required cell count based on the recommended cell density in Table 1 and the electric trochanter used (for a 0.2 cm electric trochanter, the volume is calculated as 0.1 ml; for a 0.4 cm electric trochanter, the volume is calculated as 0.25 ml.). If a 0.2cm electroconvulator requires a cell density of 5×10^6 cells/ml, then each electroconvulator requires 0.1ml * 5×10^6 cells/ml=0.5×10^6 cells.

For suspended cells, the cell density during electroporation is generally 10×10^6 cells/ml.

For adherent cells, the cell density during electroporation is generally $1\text{-}5\times10^6$ cells/ml.

3.2. Restore the EZ Transfer transfection solution to room temperature, preheat trypsin EDTA and complete culture medium to 37 ℃. Pre add the complete culture medium to the culture vessel to prepare the cultured cells after electroporation.

3.3. Routine collection of cells, centrifugation, removal of supernatant, resuspend cells in a certain amount of complete culture medium according to cell dosage, count, measure cell density, and calculate the required cell volume.

3.4. Transfer the corresponding number of cells to a new test tube using a pipette, and then centrifuge at room temperature for 10 minutes at a speed of 90 ×g. Completely remove the supernatant.

3.5. Resuspension cells with an appropriate amount of EZ Transfer. For a 0.2cm electric transfer cup, 0.1ml of EZ Transfer is required each time, while for a 0.4cm electric transfer cup, 0.25ml of EZ Transfer is required each time.

3.6. Add nucleic acid to the EZ Transfer transfection solution/cell mixture solution to prepare the EZ Transfer transfection solution/nucleic acid/cell mixture solution. The volume of nucleic acid should generally not exceed 10% of the amount of electroporation solution used (0.1ml of electroporation solution should not exceed 10μl, 0.25ml of electroporation solution should not exceed 25μl).

3.7. Transfer the cell/nucleic acid/electroporation solution into an electroporation cup and perform electroporation.

Note: 1) After mixing cells with electroporation buffer, electroporation should be performed as soon as possible.

2) During electric rotation, bubbles should be avoided in the electric rotor cup.

3.8. Immediately transfer the electroconverted cells to a culture dish and incubate for 4-48 hours to analyze the results.

Common problems and solutions

Problem	Solution
Low efficiency of electrotransfection	
Poor cell density during electrotransfection	Determine the optimal cell density for each cell type to maximize the efficiency of electroporation. In subsequent experiments, the reproducibility of the experiment was tested based on this cell density. For most suspended cells, it is recommended to have a cell density of 10 × 106 cells/ml during electroporation. For adherent cells, it is recommended to have a cell density range of 1-5 × 106 cells/ml.
Cells are not in the exponential growth phase during electrotransfection	Cell passage should be performed 1-3 days before electroporation to ensure active cell division and optimal cell density during electroporation.
Poor DNA concentration and purity	It is recommended to use plasmid DNA with an OD value of 1.8-2.0. The optimized range of DNA concentration in the working solution is between 5-50ug/ml. Generally, the experiment can start at 20ug/ml.
Poor quality of plasmid DNA	1. Use high-purity, sterile, endotoxin free, and pollution-free DNA. 2. Purify DNA using cesium chloride gradient or anion exchange. 3. Do not use DNA prepared using miniprep kits as it may contain high levels of endotoxins. 4. Do not use ethanol precipitation method to purify DNA. The high residual salt produced by ethanol precipitation can alter the current and be harmful to electroporation.
Incorrect vector sequence	Verify if the expression system of plasmid DNA is correct.
Lack of correct control group in the experiment	Set up a positive control group using EZ Transfer electroporation solution, such as transfection with luciferase β- Plasmids encoded by galactosidase or green fluorescent protein (gfp).
Poor siRNA concentration	The optimal siRNA concentration is generally between 250 and 750 nM final concentration. The recommended starting concentration is 250nM.
Incorrect siRNA sequence	Ensure that the sequence of siRNA is correct. It is best to test multiple sequences for optimal interference efficiency and eliminate off target effects.
Poor quality of siRNA	Use plastic containers without RNase treatment to avoid siRNA degradation. Before the experiment, use PAGE electrophoresis to detect whether siRNA is degraded.
The experiment lacks the correct siRNA experimental control group	1. Set up a blank control group to test the cell growth status. 2. Set up a negative control group consisting of serum-free culture medium, EZ Transfer electrotransfection reagent, and negative siRNA. 3. Set up a positive control group, using serum-free culture medium+EZ Transfer electrotransfection reagent+targeted siRNA transfection of housekeeping genes, such as GAPDH or Lamin A/C, and then detect gene expression through Western blotting or mRNA quantification.
Poor cell incubation time after electrotransfection	Determine the optimal incubation time after electrotransfection for each cell type and experiment. Can test different cell incubation times (e.g. 4-72 hours). The optimal incubation time is generally 4-48 hours.
Cytotoxicity	
Pulse intensity may be too high during electroporation	Reduce the voltage with a gradient of 10V and/or decrease the capacitance with a gradient of 100μF.

Cells did not immediately transfer to containers containing complete culture medium	After each electroporation, immediately transfer the cells from each test tube to a culture dish containing hot complete culture medium.
Plasmid DNA contains endotoxins	1. Perform electroporation using high-purity, sterile, endotoxin free, and pollution-free DNA. 2. DNA purified using cesium chloride gradient or anion exchange should not be prepared using miniprep kits as it may contain high levels of endotoxins.
DNA contains a large amount of salt ions	If DNA is prepared through ion exchange columns and ethanol precipitation steps, we recommend changing the DNA solution to a salt free or low salt solution, such as washing DNA in water and adding 5 mM NaCl.
Excessive DNA content in electroporation mixture	Reduce the amount of DNA used during electroporation. The experiment can be conducted using a DNA concentration of 5ug/ml in the final electroporation volume. Compare the toxicity level with the experimental results of the cell+EntersterTME solution control group to evaluate the effectiveness of DNA transfection. If cytotoxicity is still observed, it is likely due to the concentration of EZ Transfer/cell mixture or the dissolution of too many cells.
The expression of target genes is toxic to cells	Compare the cytotoxicity of the blank control group and the transfected empty vector control group to evaluate the toxic effect of the protein expressed by the target gene on cells. If electroporation experiments require lower levels of target gene expression, it may be considered to reduce the amount of plasmid used. If necessary, transfect using vector DNA (such as empty clone vectors) while maintaining the optimal final DNA concentration (20ug/ml).
Essential genes are disrupted	If siRNA used for electrotransfection directly acts on essential genes in cells, it may lead to cytotoxicity due to gene knockdown. Set up a negative control group experiment to compare the cytotoxicity levels of the negative siRNA control group and the knocked down genome.
Changes in cell morphology	Mycoplasma contamination can alter cell morphology and affect transfection efficiency. Check for Mycoplasma contamination in cells. Fresh frozen cells or appropriate antibiotics can be used to clear Mycoplasma. Excessive or insufficient cell passages can make cells more sensitive to electroporation. Maintain the same number of cell passages between experiments to achieve reproducibility.

Related products

TR2000: Used for transfecting DNA into HEK293T cells.