TransXG™ Transfection Reagent

User's Instruction

Description

TransXG™ Transfection Reagent is an efficient and low toxicity transfection reagent, which is made from biodegradable polycations and other components. It is suitable for the transfection of a variety of mammalian cells, especially for the transfection of adherent cells. Compared with traditional liposome transfection reagents, TransXG™ has great advantages in stability, transfection efficiency, and experimental operation.

TransXG™ Transfection Reagent has high transfection efficiency, good repeatability, and simple operation for most adherent cells. Transfection reagents and plasmid DNA can form nanoparticles within 5 min at room temperature. The formed DNA nanocomplexes can be directly added into the complete cell culture medium without the need to remove the transfection reagents and DNA complexes by replacing the culture medium to reduce the cytotoxicity of the transfection agent, nor the need to use specific transfection-specific media such as MEM. The control experiment showed that the transfection effect of this product was not affected by serum and antibiotics in the culture medium, and the best transfection efficiency could be obtained by simply adjusting the ratio of transfection reagent and plasmid DNA.

This reagent has obtained ideal results in the transfection experiments of HEK293, HeLa, MCF-7, COS-7, CHO, HepG2, NIH-3T3, L929 and 4T1 cells.

Protocol

Other reagents required: Users need to prepare 0.15 M NaCl (double-distilled water preparation, high pressure, or filtration sterilization) as transfectant and DNA diluent.

1. Preparation of transfected cells

Adherent cells: one day (24 h) before transfection, inoculate $1x10^5$ - $2x10^5$ cells into 24-well plates, and add 1 ml of complete medium to achieve 70%-90% confluence when transfected.

Note: Adherent cells can also be directly transfected by suspension cell method after passage. Transfection can also be done when cell adherence is essentially complete after passage (e.g. HeLa cells usually adhere completely within 2 h after passage). But the premise of these simplified methods is that the cell condition at passage should be very well, and overcultured cells should not be used. In addition, the culture medium inoculated with cells has been 24 h since the time of transfection, and if it

has turned yellow, it is better to replace it with fresh medium at the time of transfection.

Suspension cells: Collect suspension cells by centrifugation and resuspend the cells with fresh medium and inoculate into 24-well plates with $1x10^5$ - $2x10^5$ cells per well and 1 ml complete medium.

2. **Preparation of transfection complexes**

   For most cells, the ratio of plasmid DNA (μg) to TransXG™ Transfection Reagent (μl) is roughly 1:3-1:5. In two sterile centrifuge tubes, add 50 μL 0.15 M NaCl each. Add plasmid DNA into one tube. Gently mix to make a DNA dilution. TransXG™ Transfection Reagent diluent is prepared by adding TransXG™ Transfection Reagent into the other centrifuge tube and mixing gently.

   Note: The most suitable amount of DNA and transfection reagents needs to be optimized according to different cells and different media. For 24-well plates, the dosages in each hole are generally within the range of 1-2 μg and 3-5 μL, respectively. Under the same transfection efficiency, the amount of transfectant should be as little as possible.

3. Add 50 μL TransXG™ Transfection Reagent dilution to 50 μL DNA dilution and gently mix with a pipette. The transfection complexes are obtained by placing them at room temperature for 5 min.

4. Add the prepared transfection complex directly to the cell culture medium and shake the plate gently to mix the complex. The transfection complex can be directly added to the cell culture medium containing serum.

5. Incubate plates in a 37℃, 5% CO_2 incubator, and downstream experiments could be carried out after 24-72 h.

6. If want to screen stable cell lines, dilute the cells at a ratio of 1:10 or higher after 24 h of transfection, passage to other culture plates, and the next day add appropriate concentrations of drugs (such as G418, Zeocin, etc.) for screening.

Optimization

There are many factors that affect the transfection efficiency, such as the properties and conditions of the cells, the amount of transfection reagents, the amount of transfected DNA, and the transfection reagent/DNA ratio, the shape and size of the formed complex, the cell number/cell density ratio, the incubation time of the cell and the transfection complex. The optimal transfection conditions should be optimized in specific practice.

After optimization in a 24-well plate, the same conditions can be used for the same cell line.

If different cell culture plates are used for transfection, the optimized transfection system can be scaled up or down according to the table below.

Surface area comparison of different cell culture plates

Plate	96-well	48-well	24-well	12-well	6-well	35mm	60mm	100mm
Area (cm^2)	0.3	0.7	2	4	10	10	20	60
Ratio to 24-well	0.2	0.4	1	2	5	5	10	30

Problems and Solutions

Low transfection efficiency

1. Plasmid concentration is too low - suggestion: use the appropriate amount of plasmid.

2. The purity of plasmids is too low – suggestion: use high quality plasmids ($OD_{260}/OD_{280} \geq 1.8$).

3. Poor cell growth – suggestion: ensure that cell density and morphology are good.

4. Reduce the volume of culture medium by half (after 4 h of transfection, add sufficient culture medium).

5. Starting from the initial dosage, adjust the dosage of DNA and TransXG™ Transfection Reagent in the prepared transfection solution (keep the total volume of the transfection working solution unchanged) to determine the optimal transfection conditions of different cells. To do this, usually you can mix the amount of fixed DNA (such as 2 μg) with the TransXG™ of series content, and the optimal amount of TransXG™ is determined. You can also fix the amount of TransXG™ (such as 2 μL), then mix with the DNA of series content, and the optimal amount of DNA is determined. Or you can fix the ratio of TransXG™/DNA, and then adjust the amount of plasmid.

6. Use positive control, such as plasmid around 1/10 of the total amount of transfected plasmid expressed by EGFP or beta galactosidase gene

Too much toxic on cells

1. Before inoculation step, cell growth will directly affect cell activity.

2. Cell density should not be too low when transfected.

3. Increase the volume of culture medium during transfection or reduce the amount of TransXG™ Transfection Reagent while maintaining the TransXG™/DNA ratio.

4. For some sensitive cell lines, the transfection complexes should be removed after 4 h of transfection, replaced with fresh complete culture medium.

5. Examine whether the gene product is toxic.

Storage

Stored at 2-8℃ for one year and -20℃ for two years.