

293Transfer

Product Number: TR1804

Shipping and Storage

This product is transported at room temperature and stored for a long time at 4°C, with a validity period of 12 months.

This product is safe to use and no biological or chemical toxicity has been found. If accidentally contaminated, rinse with clean water.

Description

293Transfer is a nano polymer transfection reagent developed and synthesized by our company. This reagent is synthesized using nanotechnology and is the latest generation of transfection reagents. Due to the application of nanotechnology, the transfection efficiency of 293Transfer in HEK293T cells can reach over 95%, while also exhibiting low cytotoxicity.

Note

1. Antibiotics can be added to this product during the transfection process, and the addition of antibiotics does not affect the transfection efficiency and toxicity.
2. If Trizol is needed to extract RNA after transfection, it is recommended to change the solution 6 hours after transfection.

Protocol (Taking a 6-hole plate as an example)

1. Cell laying one day in advance

Plant the cells on a 6-well plate one day in advance, with a cell density of around 50% during transfection.

2. Transfection process

2.1. Dilute 5 μ g of DNA with 50 μ l of serum-free diluent, mix well, and prepare a DNA diluent.

Note: It is recommended to use OPTI-MEM or serum-free DMEM for serum free diluents.

2.2. Dilute 5 μ l of 293Transfer with 50 μ l of serum-free diluent, mix well, and prepare 293Transfer diluent. Let it stand at room temperature for 5 minutes.

2.3. Add 293Transfer diluent to DNA diluent separately, mix thoroughly (shake with an oscillator or blow and aspirate more than 10 times with a sampler), and let it stand at room temperature for 15-30 minutes. The preparation of transfection complexes has been completed.

Note: A 30 minute soak time is more efficient than 15 minutes for transfection.

2.4. Add the transfection complex to the culture container containing cells and complete culture medium, and gently mix well.

Note: 1)For this reagent, using serum containing full culture medium can help improve transfection efficiency.

2)Antibiotics can be added to the complete culture medium.

2.5. After 4-6 hours of cultivation, replace the culture medium and continue to cultivate for 24-48 hours.

Note: If the cells have no toxicity or other adverse conditions, there is no need to change the culture medium 4-6 hours after transfection.

Transfection dosage in different cell culture containers

| Cell culture container | Surface area (cm ²) | Ratio of surface area to 24 well | The amount of DNA added per well* | Volume of DNA dilution solution per well | 293 Transfer usage per hole* | 293Transfer diluent volume, total amount of culture medium per well | Total amount of culture medium per well |
|------------------------|---------------------------------|----------------------------------|-----------------------------------|--|------------------------------|---|---|
| 96-well | 0.3 | 0.2 | 0.25 μ g | 5 μ l | 0.25 μ l | 5 μ l | 100 μ l |
| 48-well | 0.7 | 0.4 | 0.5 μ g | 10 μ l | 0.5 μ l | 10 μ l | 200 μ l |
| 24-well | 1.9 | 1 | 1 μ g | 25 μ l | 1 μ l | 25 μ l | 500 μ l |
| 12-well | 3.8 | 2 | 2 μ g | 25 μ l | 2 μ l | 25 μ l | 1ml |



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| | | | | | | | |
|------------------|----|----|------|-------|------|-------|------|
| 6-well/35-mm | 10 | 5 | 5µg | 50µl | 5µl | 50µl | 2ml |
| 60 mm/T25 flask | 21 | 10 | 10µg | 125µl | 10µl | 125µl | 5ml |
| 100 mm/T75 flask | 58 | 30 | 25µg | 500µl | 25µl | 500µl | 15ml |

Note: If used for simultaneous co transfection of multiple plasmids, the amount of DNA used refers to the total amount of each plasmid used. In order to achieve higher transfection efficiency, it is recommended to increase the amount of DNA and transfection reagents by 2-4 times simultaneously.

Optimization

Due to the ratio of DNA to transfection reagent dosage being an important factor determining transfection efficiency, as well as the differences in plasmid quantification errors, plasmid purification levels, and cell states among different laboratories, there are differences in the optimal experimental conditions for different cells and laboratories. To achieve the highest transfection efficiency, it is recommended to optimize the initial application first. After determining the optimal conditions, the experimental results will be very stable. The following table lists the optimization schemes in 6-well for reference.

6-well optimization plan

| | 1 | 2 | 3 |
|--------------------------------------|-------|-----|-----|
| The amount of DNA per well | 1.5µg | 3µg | 5µg |
| Dosage of transfection test per well | 1.5µl | 3µl | 5µl |

According to the optimized conditions of the pre experiment, apply it to other culture containers in proportion to the surface area of the culture vessel.

Common problems and solutions

| Problem | Reason | Solution |
|---|---|---|
| Low efficiency after 24 hours of transfection | Cell density too high | Suggest reducing the confluence to 30-50% during transfection and extending the observation time after transfection |
| | Insufficient cultivation | Suggest reducing the cell confluence during transfection and observing the results for 48 hours or even longer |
| | Low DNA purity | It is recommended to use OD260/OD280 at around 1.8, without protein and RNA, and without endotoxin DNA |
| | Poor ratio of DNA to transfection reagents | Suggest pre experimental optimization |
| | Dilution solution containing serum or protein | Suggest using OPTI-MEM or serum-free DMEM dilution |
| Cytotoxicity | The plasmid expression system is highly toxic | Suggest using other reliable plasmids as positive controls to compare transfection results |
| | Cell density too high | Suggest reducing the number of cells |
| | Poor ratio of DNA to transfection reagents | Suggest pre experimental optimization |
| | Cellular contamination | Suggest thoroughly cleaning all cell culture related products |

Other related reagents

TR1000: Transfect small fragments of RNA (siRNA, miRNA, mimic, inhibitor, etc.) into animal cells.

TR1812, TR1811: Animal in vivo transfection of RNA and DNA.

TR3011: Virus infection enhancement series reagents can enhance the infection efficiency of lentiviruses, adenoviruses,

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adeno-associated viruses, and retroviruses.