Tinzyme Co., Limited

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TRANS2000 transfection reagent

Product Number: TR2000

Shipping and Storage

This product is transported at room temperature and stored for a long time at 4℃, 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

TRANS2000 transfer reagent is a nano polymer transfection reagent developed and synthesized by our company. This reagent is synthesized using nanotechnology and is the latest generation of non viral transfection reagents. Due to the application of nanotechnology, TRANS2000 transfer reagent exhibits excellent low toxicity and high efficiency performance in cell transfection processes.

This product is recommended for transfection of conventional DNA and long segments (>1kb) of mRNA and other RNA.

Protocol (Taking a 24 hole plate as an example)

1. Cell laying one day in advance

   Plant the cells in a 24 well plate one day in advance, with a cell density of around 60% during transfection.

   Antibiotics can be added to this product during the transfection process. The addition of antibiotics does not affect the transfection efficiency and toxicity. If there are no special requirements, it is recommended to add antibiotics.

2. Transfection process

   2.1. Dilute 0.8μg of nucleic acid with 25μl of serum-free diluent, mix well, and prepare a nucleic acid diluent.

   Note: It is recommended to use OPTI-MEM, serum-free DMEM, or 1640 for serum-free diluents.

   2.2. Dilute 2μl of TRANS2000 transfer reagent with 25μl of serum-free diluent, mix well, and prepare a TRANS2000 transfer reagent diluent. Let it stand at room temperature for 5 minutes.

   2.3. Add the TRANS2000 transfer reagent diluent to the nucleic acid 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 minutes. The preparation of transfection complexes has been completed.

   2.4. Add the transfection complex to the culture container containing cells and complete culture medium, gently mix well, and place it in the incubator for cultivation.

   Note: 1) For this reagent, only serum-free liquid should be used during the dilution process. For other cell cultures, it is recommended to use a complete culture medium containing serum, which can help improve transfection efficiency.

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

Transfection dosage in different cell culture containers

Cell culture container	Surface area (cm^2)	Ratio of surface area to 24 well	The amount of nucleic acid added per well*	Volume of nucleic acid dilution solution per well	TRANS2000 transfection reagent volume*	TRANS2000 transfection reagent dilution volume	Total amount of culture medium per well
96-well	0.3	0.2	0.2μg	10μl	0.5μl	10μl	100μl
48-well	0.7	0.4	0.32μg	15μl	0.8μl	15μl	200μl
24-well	1.9	1	0.8μg	25μl	2μl	25μl	500μl
12-well	3.8	2	1.6μg	25μl	4μl	25μl	1ml
6-well/35-mm	10	5	4μg	50μl	10μl	50μl	2ml
60mm/T25	21	10	8μg	125μl	20μl	125μl	5ml

flask							
100 mm	58	30	24μg	500μl	60μl	500μl	15ml

2.5. After 4-6 hours of observation, if the cells have no adverse effects such as toxicity, continue to culture. DNA expression can be detected after 24-48 hours, and mRNA can be detected after 6-48 hours according to the experiment.

Note: 1) If cells experience growth arrest, death, and other adverse conditions, as well as the need for Trizol to extract RNA, the culture medium needs to be changed 4-6 hours after transfection.

2) 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 1.5-2 times simultaneously.

3) If used for transfection of long segments (>1kb) mRNA and other RNAs, please pay attention to removing RNA enzymes in liquids, containers, tips, etc. during the operation, and strictly prevent RNA enzyme damage. The detection time of the results can be shortened to 6-24 hours after transfection or extended to 48 hours according to specific experimental conditions.

Optimization

Due to the ratio of nucleic acid 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

Number	1	2	3	4
The volume of nucleic acid per well(μg)	2μg	3μg	4μg	4μg
The volume of transfection test per well(μl)	5μl	7.5μl	8μl	10μl

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 fusion degree to 50% during transfection and extending the observation time after transfection
	Insufficient cultivation time after transfection	Suggest reducing the cell confluence during transfection and observing the results for 48 hours or even longer
	Low nucleic acid purity	It is recommended to use OD260/OD280 at around 1.8, without protein and RNA, and without endotoxin nucleic acid
	Poor ratio of nucleic acid to transfection reagents	Suggest pre experimental optimization
	Dilution solution containing serum or protein	Suggest using OPTI-MEM, serum-free DMEM, or 1640 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 nucleic acid 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.

TR3015: Animal in vivo transfection of RNA and DNA.

TR3091: Virus infection enhancement series reagents can enhance the infection efficiency of lentiviruses, adenoviruses, adeno-associated viruses, and retroviruses.