Tinzyme Co., Limited

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TRANS1000 transfection reagent

Product Number: TR1000

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

TRANS1000 transfer reagent is a nano polymer transfection reagent developed and synthesized by our company. This reagent is synthesized using nanotechnology and is a new generation of non viral transfection reagents. Due to the application of nanotechnology, the TRANS1000 transfer agent exhibits excellent low toxicity and high efficiency performance during cell transfection.

This product is recommended for transfecting small fragments of nucleic acid (RNA or DNA below 1kb) such as siRNA, microRNA (miRNA), mimic, inhibitor, etc. into animal cells (including various cell lines, primary cells, suspension cells, insect cells, etc.).

This product has demonstrated good RNA transfection efficiency in various cell lines and low cytotoxicity, with lower cytotoxicity being particularly important for RNAi experiments.

Note

1. Due to the low cytotoxicity of this product, the number of cells transfected with this product is relatively small, which helps to improve transfection efficiency.
2. Using this product for transfection and measuring by mass (μg), for 21nt double stranded siRNA, 1OD=3.0nmol=40μg.
3. For larger nucleic acids larger than 1kb, please use our company's TRANS2000 transfer reagent long nucleic acid transfection reagent.
4. If Trizol is needed to extract RNA after transfection, please change the solution 6 hours after transfection.

Protocol (Taking 24 well plate siRNA transfection as an example)

1. Planting cells one day in advance
   - 1.1. Adherent cells: Plant the cells in a 24 well plate one day in advance, with a cell confluence of about 30% during transfection. The total amount of culture medium before transfection is 0.45ml.
   - 1.2. Suspended cells: Transfection experiments were conducted using logarithmic growth phase cells, which were one-third of the number of conventional cultured cells. If the number of cells in conventional culture for a certain cell is 6×10^5, then transfect with 2×10^5 cells.
2. Transfection process
   - 2.1. Take 0.67μg (50pmol) of siRNA, add a certain amount of serum-free diluent, mix well, and prepare an RNA diluent with a final volume of 25μl.

     Note: It is recommended to use OPTI-MEM, serum-free DMEM, or 1640 for serum-free diluents.
   - 2.2. Take 1μl of TRANS1000 transfer reagent, then add 24μl of serum-free diluent, mix well, and prepare a TRANS1000 transfer reagent diluent with a final volume of 25μl. Let it stand at room temperature for 5 minutes.
   - 2.3. Mix the TRANS1000 transfer reagent diluent and RNA diluent 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. Drop 50μl of transfection complex onto cells containing 0.45ml of full culture medium (which can contain 10% serum and antibiotics), move the culture dish back and forth, and mix evenly.

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

2.5. Observe the cell status 6 hours after transfection. If the condition is good, there is no need to change the culture medium. Continue to culture for 24-96 hours to obtain the results.

Note: 1) After siRNA transfection, continue to culture for 24-72 hours to obtain results at the mRNA level, and continue to culture for 24-96 hours to obtain results at the protein level.

2) In some laboratories, due to differences in serum and culture conditions, a small amount of black dot like precipitate may appear in the culture medium under the microscope after transfection. It is a protein binding product in the transfection reagent and serum, and does not affect the transfection result and cell state. It can be removed by changing the medium.

Table 1 Recommended transfection dosages for different cell culture containers

Cell culture container	Surface area (cm^2)	Total amount of culture medium	Dilution volume	Small nucleic acid dosage	TRANS1000 transfection reagent
96-well	0.3	100μl	10μl	0.15μg	0.25μl
48-well	0.7	200μl	15μl	0.3μg	0.5μl
24-well	1.9	500μl	25μl	0.67μg	1μl
12-well	3.8	1ml	25μl	1.33μg	2μl
6-well/35-mm	10	2.5ml	50μl	3.33μg	5μl
60 mm/T25 flask	21	5ml	125μl	6.67μg	10μl
100 mm	58	15ml	250μl	20.0μg	30μl

Optimization

Due to differences in RNA sequence, synthesis conditions, and the presence or absence of fluorescent markers, RNA and transfection reagents will have different optimal conditions under different conditions. It is recommended to conduct preliminary experimental optimization first. The following table lists the optimized siRNA transfection schemes at 24well for reference.

Optimization of RNA concentration and transfection reagent quantity (24well)

	1	2	3	4
RNA working concentration	25nM	50nM	100nM	150nM
Amount of RNA per well (pmol)	0.17μg(12.5pmol)	0.33μg(25pmol)	0.67μg(50pmol)	1μg(75pmol)
Dosage of transfection test per well	0.25μl	0.5μl	1μl	1.5μl

According to the optimized experimental results, the ratio of fixed RNA (μg) to transfection reagent amount (μl) was applied to other culture containers in proportion to the surface area of the culture vessel.

Common problems and solutions

Problem	Reason	Solution
Silence phenomenon is not obvious after transfection	Poor ratio of nucleic acid to transfection reagents	Pre experimental optimization
	Poor cell density	Adjust cell density to a fusion degree of 20-40% during transfection
	Low nucleic acid efficiency	Select the optimal nucleic acid and use known efficient RNA as a control
	RNA enzyme contamination	Suggest materials and experimental equipment such as pipettes, centrifuge tubes, and solutions that are free of RNA enzymes and endotoxins.
	Insufficient cultivation time after transfection	At the mRNA level, the results can be validated after 48 hours, and at the protein level, the results can be validated after 72 hours

Cytotoxicity	Poor ratio of nucleic acid to transfection reagents	Pre experimental optimization
	Poor cell density	Adjust cell density to a fusion degree of 20-40% during transfection
	Toxicity of culture medium	Using a full culture medium containing 10-20% serum
	Cellular contamination	Suggest thoroughly cleaning all cell culture related products

Other related reagents

TR2000: Transfect long nucleic acids into animal cells.

TR1812, TR1811: 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.