Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

EVO Transfer, RNA

Product Number: TR1811

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

EVO Transfer, RNA 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, EVO Transfer, RNA exhibits excellent low toxicity and high efficiency during cell transfection. This product is used for in vivo transfection of RNA.

Features

The method is simple and fast, with no obvious inflammatory response to animals, and is safe for operators.

Application

1. In vivo studies of gene therapy in animals
2. In vivo studies on RNA interference in animals
3. In vivo study of protein function in animals

Note

1. The preparation of transfection complexes is carried out at room temperature. Before operation, please restore the nucleic acid, transfection reagents, diluents, etc. to room temperature. Low temperature operation, long-term storage, minimal or no use of diluents, and mixing nucleic acids and transfection reagents in large reaction systems may cause the aggregation of transfection complexes and result in turbidity. In general, slight turbidity does not affect the transfection effect. If turbidity needs to be avoided, it is recommended to operate at room temperature, ready to use, using diluents, and avoiding large amounts of preparation at once.
2. RNA dose. The initial dose for intravenous administration of nucleic acid is 2.5mg/kg. The recommended dosage is 2.5-5mg/kg. The higher the dosage, the better the effect, with the standard of not causing animal inflammation and death. The following table shows some common dosages and volumes of administration.

Table 1. Dosage and volume of administration

	Administration route	Nucleic acid dosage	Maximum administration volume
Adult mice	Caudal vein	50-150μg	200μl-600μl
	Ventricle	1-2.5μg	5μl
	Peritoneum	100μg	0.6ml-1ml
	Testis	3-5μg	10μl
nude mice	Subcutaneous tumor	10-50μg	100μl
Adult rats	Ventricle	2-5μg	20μl
	Caudal vein	500μg-2.25mg	1-2ml
Adult rabbit	Pulmonary and tracheal perfusion	300-700μg	300-700μl

Note: In general, for 21nt double stranded siRNA oligo, 1OD duplex=3.0nmols=40μg. But there are also some companies that synthesize double stranded siRNA oligos, with 1OD duplex=33μg. Please consult the relevant synthesis company for details.

3. Requirements for nucleic acid testing. It is recommended to dissolve with pure water and ensure high purity and endotoxin free. The recommended concentration of nucleic acid is 1-4μg/μl. Nucleic acid diluted with non pure water (such as PBS) may cause precipitation. The purity of nucleic acid will greatly affect the transfection efficiency, so it is necessary to use high-purity and high-quality nucleic acid.
4. The amount of EVO Transfer, RNA used. In general, nucleic acid (μg) and EVO Transfer, RNA (μl) are used in a 2:1 ratio.
5. Tail vein injection: To maintain the working concentration of the transfection complex and blood isotopes during injection, it is necessary to dilute with solutions such as glucose (preferred) and physiological saline.

The following table shows the composition of each component (nucleic acid concentration 1μg/μl) when a tail vein injection of 20g body weight mice was administered at a dose of 200μl at a dose of 2.5mg/kg.

Table 2. Composition of 200μl transfection complexes

200μl transfection complex	Nucleic acid diluent	50μl (50μg)nucleic acid
		50μl of 10% glucose solution
	Transfection reagent diluent	25μl transfection reagent
		50μl of 10% glucose solution
		Pure water 25μl for replenishment

6. Local injection: First, determine the maximum volume that needs to be finally injected. After determining the maximum volume, the dosage is determined based on the ratio of nucleic acid (μg): transfection reagent (μl)=2:1. For example, when the local injection volume is 30μl, the composition of each component is determined, with a nucleic acid concentration of 1μg/μl (dissolved in pure water). The amount of dilution solution can be diluted with glucose solution or physiological saline during local injection, and the dosage can be appropriately reduced or even not used. However, the reduction of dilution solution may cause aggregation of transfection complexes, which need to be injected as soon as possible.

Table 3. Composition of Local Injection 30μl Transfection Complex

30μl Transfection complexes	Nucleic acid solution	15μg(15μl)nucleic acid
	Diluted solution	7.5μl
	Transfection reagent	7.5μl Transfection reagent

Protocol

Taking 50μg of nucleic acid and 25μl of transfection reagent, with a total injection volume of 200μl, 20g of mouse tail vein injection as an example for explanation.

1. **Dilution of nucleic acid.** Dilute 50μg of nucleic acid with an appropriate amount of endotoxin free pure water to 1μg/μl, add a 10% glucose solution (w/v) of 50μl to achieve a final glucose concentration of 5% and a final volume of 100μl, and mix well.
2. **Dilution of transfection reagents.** Dilute 25μl of EVO Transfer, RNA reagent with 50μl of 10% glucose solution and supplement with 25μl of pure water to obtain a final glucose concentration of 5% and a final volume of 100μl of liquid. Mix thoroughly.
3. **Transfection complex formation.** Add the diluted transfection reagent to the diluted nucleic acid solution at room temperature and immediately shake thoroughly to mix well.
4. Let it stand at room temperature for 15 minutes. The prepared transfection complex should be prepared and used immediately, and should not be stored for a long time.
5. **Animal injection.**

Explanation: 1) In some cases, due to the amount of nucleic acid and transfection reagents used, it may not be possible to maintain a glucose concentration of 5% (or 0.9% of physiological saline) in the injection solution. In this case, it is only necessary to ensure that nucleic acid (μg): transfection reagent (μl)=2:1, and that the amount and final concentration of glucose solution increase or decrease moderately, without affecting transfection efficiency.

2) Generally speaking, local injection of drugs targeting target organs has a more significant effect, less dosage, and is easier to operate than tail vein injection;

3) When injecting into the tail vein, please master the injection technique. Generally, the distal one-third of the

vein should be used for injection. If resistance or slight protrusion is felt, please stop the injection and search for a new vein for operation. Do not forcefully push the injection, otherwise it is easy to inject the medication into the tail, causing the tail to ulcerate. After the injection is completed, remove the needle and press the needle hole for more than 10 seconds to prevent the medication from flowing out.

4) A dosage of 2.5mg/kg is the initial dosage, and if the animal can tolerate it, the dosage can be increased proportionally for better results.

5) Local injection, injecting as much medication as possible, is beneficial for improving the transfection effect.

6. **Gene expression detection.** Generally speaking, based on the differences in injection methods and target organs, the gene expression effect is better after 12-48 hours.

Explanation: 1) It is recommended to first use qRT PCR to detect the transfection effect at the molecular level;

2) If protein level detection is required, Western blot method is generally used. At the tissue and organ level, immunohistochemistry method can be used.

7. **Long term administration.** The optimal time for a drug administration test is 12-48 hours after injection. If long-term effects need to be maintained, multiple injections can be used, with an injection frequency generally every 2-3 days, or it can be appropriately extended to every 7 days according to experimental conditions.

Common problems and solutions

Problem	Reason	Solution
The transfection complex shows precipitation	Dissolve nucleic acid with TE or other substances	Dissolve nucleic acid in pure water
	Mixing nucleic acid and transfection reagents at low temperatures	Nucleic acid and transfection reagents should be mixed at room temperature
	Insufficient amount of diluent	Add diluent according to instructions
	The final concentration of nucleic acid during injection is too high	The final concentration of nucleic acid during injection is<=0.5μg/μl
	Transfection complexes left for too long	Transfection complexes are best prepared and used immediately
	The volume of the prepared transfection complex is too large	Suggest starting from a small volume to prepare transfection complexes
Low transfection level	Test method	It is recommended to use qRT PCR upstream method to determine transfection efficiency before using other downstream detection methods.
	Low dosing	Increase the dosage of medication without causing animal death
Animals with inflammatory reactions or death	Injection operation error	Inject the medication correctly into the corresponding area
	Excessive nucleic acid dosage	Reduce the amount of nucleic acid used, maintain the ratio of nucleic acid to transfection reagents, and simultaneously reduce the amount of transfection reagents used.
	Injection contains endotoxins	Using endotoxin free nucleic acid
		Before injection, use a 0.22um filter to filter and sterilize

Related products

TR2000: Used for transfecting DNA into HEK293T cells.

TR1000: Used for in vitro transfection of RNA into animal cells.