Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

TurboFect™ *in vivo* Transfection Reagent

120 µl (for 20 transfections using 50 µg DNA)

#R0541
Lot:
Expiry Date:

Store at 4°C

ISO 9001 | ISO 14001
www.fermentas.com

Description

TurboFect™ *in vivo* Transfection Reagent is a sterile solution of a cationic polymer in water. The polymer forms compact, stable, positively charged complexes with DNA. These complexes protect DNA from degradation and facilitate gene delivery *in vivo*. Reagent causes no detectable inflamatory response and is suitable for DNA administration via various routes, including intravenous, intraperitoneal, intratumoral injection, etc.

Reagents to be Supplied by the User

Sterile 5% w/v glucose solution for dilution of DNA.

General Considerations

DNA Quality Requirements

DNA quality is critical for successful transfection. An A_{260}/A_{280} ratio of 1.8 or higher is recommended. DNA should be sterile and free of any contaminants such as endotoxins.

Choice of promoter

High gene expression depends on both the promoter under which the gene of interest is expressed and the targeted tissue/organ. Cytomegalovirus (CMV) promoter is best known for high gene expression in a wide variety of cells. Some researchers prefer LTR, simian virus (SV40) or Rous sarcoma virus (RSV) promoters.

Transfection Reagent/DNA Ratio

The volume of transfection reagent used depends on the amount of DNA, transgene and animals to be transfected. The ratios presented in the protocols below are starting amounts and can be further optimized for best results.

Amount of DNA and suggested injection volume

The amount of DNA and suggested injection volume depend on the experimental animal and the route of administration (see Table 1) as well as the targeted tissue or organ and the expression vector.

To prevent precipitation of TurboFect™/DNA complexes, the final concentration of DNA in the injection mix should not exceed 0.5 µg/µl.

General Protocol for ***in vivo*** transfection

The protocol is optimized for transfection of mice via the tail vein.
Quantities and volumes should be scaled-up according to the animal to be transfected (see Table 1).

1. Dilute 50 µg of DNA in 400 µl sterile 5% glucose solution. Vortex gently and spin down briefly.
2. Briefly vortex TurboFect™ *in vivo* Transfection Reagent and add 6 µl of it to the diluted DNA. Mix immediately by pipetting or vortexing.
3. Incubate for 15-20 min. at room temperature.
4. Perform injections.
5. Monitor gene expression with the method most suitable for your studies.

Note

The A_{260}/A_{280} ratio should be at least 1.8 for purified DNA. It is important to use endotoxin-free DNA (less than 0.1 EU/1 µg DNA).
The amount of DNA and suggested injection volume depend on the experimental animal and the route of administration (see Tables 1 and 2) as well as on the targeted tissue or organ and the expression vector.

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Table 1. Suggested amount of DNA and injection volume

Animal	Route of injection	Suggested amount of DNA (µg)	Suggested injection volume
Adult Mouse	Intravenous (tail)	50	400 µl
	Intraperitoneal	100	800 µl
	Intratumoral	10	50 µl
	Brain	2.5	5 µl
Newborn mouse	Brain	1	2 µl
Nude mouse	Intravenous (tail)	50	400 µl
	Intratumoral	10	50 µl
Adult rat	Intravenous (tail)	500	2 ml
	Brain	20	25 µl
Adult rabbit	Intravenous	5000	10 ml

Table 2. Scale-up ratios for transfection using TurboFect™ ***in vivo*** Transfection Reagent

Amount of DNA (µg)	Volume of TurboFect™ ***in vivo*** Transfection Reagent (µl)	
	Recommended	Range
1	0.12	0.1-0.16
5	0.6	0.5-0.8
10	1.2	1-1.6
50	6	5-8

QUALITY CONTROL

Transfection efficiency was tested on BALB/c female mice (7-8 weeks old) using 50 µg Firefly luciferase coding plasmid and 6 µl of TurboFect™ *in vivo* Transfection reagents per mouse. The delivery of the transgene was evaluated by measurement of Firefly luciferase activity in total protein extracted from the lungs of transfected animals. 100% of tested mice expressed the Firefly luciferase.

Quality authorized by:  Jurgita Zilinskiene

Troubleshooting

Problem	Possible Cause and Solution
Low transfection efficiency	Suboptimal reagent/DNA ratio. Optimize the amount of transfection reagent added to the fixed amount of DNA. Suboptimal quantity of DNA. Optimize the amount of DNA used for transfection. Keep the transfection reagent/DNA ratio constant. Poor DNA quality. Use high quality endotoxin-free DNA with an A_{260}/A_{280} ratio greater than 1.8.
Mortality	Suboptimal reagent/DNA ratio. Decrease the amount of transfection reagent added to the fixed amount of DNA. Suboptimal quantity of DNA. Decrease the amount of DNA. Keep the transfection reagent/DNA ratio constant. Poor DNA quality. Use high quality endotoxin-free DNA with an A_{260}/A_{280} ratio greater than 1.8.

Note

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans.
Please refer to www.fermentas.com for Material Safety Data Sheet of the product.