Avalanche®-*in vivo* Transfection Reagent

Cat. No. EZT-VIVO-1 **Size: 200 µl** **Store at 4°C**
600 µl

DESCRIPTION

Avalanche®-*in vivo* Transfection Reagent (Hereafter "Avalanche®-*in vivo*") is an extremely powerful reagent for *in vivo* gene functional studies and RNA interference. The proprietary mixture of lipids and polymers is able to form compact complexes with nucleic acids, and move the nucleic acids into cells via endocytosis. In addition to efficiently moving nucleic acids into the cells, Avalanche®-*in vivo* is also being able to protect the nucleic acids from lysosomal degradation, which ensures the efficient release of the nucleic acids from endosomes and maximizes the function of those nucleic acids. Avalanche®-*in vivo* has been proved to be able to perform the maximum delivery of DNA, shRNA, siRNA, or oligonucleotides into different organs in the body. No significant pro-inflammatory responses have been detected using Avalanche®-*in vivo*. Avalanche®-*in vivo* can be administered via various routes, such as intravenous, intraperitoneal, and intratumoral injection etc. Avalanche®-*in vivo* Transfection Reagent is the best choice for delivering nucleic acids *in vivo*.

Features:

- Efficient delivery of DNA, shRNA, siRNA or oligonucleotides *in vivo*
- 200 µl reagent for 20 transfections in mouse
- Efficient delivery to the lung, liver, kidney, and spleen, liver, pancreas and certain types of tumors via systemic administration
- Delivery via multiple modes of administration in many species
- Avalanche-*in vivo*®/nucleic acid conjugated complexes are stable in serum for 16h
- No detectable inflammatory responses
- Efficient siRNA and plasmid DNA delivery via direct subcutaneous tumor injection (multiple tumor types)
- Reproducible results
- Applicable for plasmid DNA/siRNA co-injection
- Developed and manufactured by EZ Biosystems LLC

IN VIVO DELIVERY PROTOCOL

1.1 Before You Start:

- Nucleic acids should be prepared in low salt buffer, since high salt content in the nucleic acid preparation may lead to precipitation upon complexes formation. For DNA delivery, the best results are achieved with high quality DNA in endotoxin free ddH_2O. For siRNA, order high quality grade siRNA (PAGE or HPLC purification).
- To avoid precipitation, the nucleic acid preparation should be at high concentration (if possible for DNA 3-7 µg/µl and for siRNA 5-10 µg/µl).
- The concentration of nucleic acids in the final injection solution should not exceed 0.5 µg/µl.
- The preparation of Avalanche®-*in vivo* /nucleic acid complexes should be performed in a laminar flow hood using a 10% sterile isotonic glucose solution (w/v) provided in the kit. This is required in order to form small and stable complexes. The use of ionic buffers such as PBS or cell culture media for complex preparation should be avoided. The final concentration of glucose in the injection volume should be 5 %.
- Prior to injections, ensure that Avalanche®-*in vivo* and glucose solution are equilibrated at room temperature.

1.2 Protocol:

The amount of nucleic acids to deliver and Avalanche®-*in vivo needed should be determined according to the animal model, the administration route and the targeted organ. Recommendations for delivery of DNA, siRNA, oligonucleotides and shRNA-expressing plasmids in rodents are given in Table 1.*

- Dilute the nucleic acid using the 10% glucose stock solution (provided) and sterile water to prepare a solution of ½ the injection volume of 5 % glucose. Vortex gently or mix by pipetting up and down.
- Dilute the Avalanche®-*in vivo* using the 10% glucose stock solution (provided) and sterile water to prepare a solution of ½ the injection volume of 5 % glucose. Vortex gently and spin down.
- Add the diluted Avalanche®-*in vivo* to the diluted nucleic acid all at once, vortex gently and spin down.
- Incubate for 15 minutes at room temperature. From this time point, the complexes are stable 4 h at room temperature.
- Perform injections into animals using complexes equilibrated at room temperature.
- For siRNA and DNA immunization protocols, repeat injections up to 3 times a week if required with freshly prepared complexes each time.

- Monitor gene expression as required at the appropriate time point (6 – 72 h after the last injection) depending on the mode of injection and the targeted organ.

1.3 Example: IV injection in mouse

Preparation of 300 µl injection volume of 5 % glucose containing 50 µg of plasmid DNA and 10 µl of Avalanche®*-in vivo*

- Dilute 50 µg of DNA into 75 µl of 10% glucose; add sterile water to 150 µl, vortex gently and spin down,
- Dilute 10 µl of Avalanche®*-in vivo* into 75 µl of 10% glucose; add sterile water to 150 µl, vortex gently and spin down.
- Add the diluted Avalanche®*-in vivo* to the diluted DNA at once, vortex briefly and spin down.
- Incubate for 15 minutes at room temperature.
- Perform injections into animals using complexes equilibrated at room temperature.
- Monitor gene expression.

Table 1. Recommended conditions for most common injection routes in mice and rats

Animal	Site of injection	Complex formation	Injection volume (5% glucose)
Mouse	IV Tail vein/retro-orbital	50 (40-60) µg nucleic acid 10.0 (6.0-14.0) µl reagent in 300 µl of 5% glucose	300 µl
	IP	100 (80-120) µg nucleic acid 20.0 (12.0-28.0) µl reagent in 1 ml 5% glucose	1 ml
	Intratumoral	10 (8-12) µg nucleic acid 2.0 (1.2-2.8) µl reagent in 50 µl of 5% glucose	50 µl
	Subcutaneous (s.c)	5 (4-6) µg nucleic acid 1.0 (*0.6-1.4) µl reagent in 10 µl of 5% glucose	10 µl
	Intracerebral	1.5 (1.2-1.8) µg nucleic acid *0.3 (*0.2-*0.4) µl reagent in 5 µl of 5% glucose	5 µl
Rat	IV	150 (120-180) µg nucleic acid 30 (18-42) µl reagent in 1 ml of 5% glucose	1.0 ml
	Intracerebral	3 (2.4-3.6) µg nucleic acid 0.6 (*0.4-0.8) µl reagent in 10 µl of 5% glucose	10 µl

* Dilute Avalanche®-*in vivo* 1:4 with H_2O prior to application (4 µl reagent + 12 µl H_2O), and then use 4 times of the volume in the table for accurate pipetting.
For siRNA delivery and DNA immunization protocols, multiple injections may be required.

TROUBLESHOOTING

Observations	Comments and Suggestions
Unsatisfactory results	• Optimize the amount of nucleic acids used in the delivery assay. • Use high quality plasmid or siRNA preparation. Ensure they contain neither salt, RNA, protein or endotoxin. For plasmid DNA, OD260/280 ratio should be greater than 1.8. It is best to use DNA prepared in water. For siRNA, prefer HPLC or PAGE purified oligos. • Optimize Avalanche®-*in vivo* /nucleic acid ratio. • Check that the nucleic acid is efficient *in vitro*. • Ensure that the complexes are formed in glucose 5%. • Ensure that both nucleic acid and Avalanche®-*in vivo* are diluted in 5% glucose before mixing.
Toxicity	• Decrease the amount of nucleic acid, Avalanche®-*in vivo*, or both. • If using plasmid DNA, ensure the preparation is endotoxin-free and in water.