



TransIT[®] In Vivo Gene Delivery System Product # MR 5125, 5100

1.0 INTENDED USE

The *TransIT[®] In Vivo* Gene Delivery System, developed by Mirus Corporation, provides highly efficient, *in vivo* delivery of nucleic acids (DNA or RNA) into parenchymal cells of various organs in mice. A single injection into the tail vein delivers genetic material simply and reproducibly. Following gene delivery, the highest levels of transgene expression are found in the liver, with lower levels of expression found in many other organs including the spleen, kidney, lungs, and heart.

2.0 FREQUENTLY ASKED QUESTIONS

2.1 The *TransIT[®] In Vivo* Gene Delivery System seems to be a very rigorous protocol. How does a mouse behave after a successful injection?

Five minutes after a successful injection with *TransIT[®] In Vivo* Gene Delivery System, the behavior patterns of the mice return to normal. Their breathing, eating, drinking and sleeping patterns are normal. Long term studies show that the mice experience a normal life span with no indications of adverse long-term effects such as growth rate or changes in occurrence of tumors.

2.2 Can the *TransIT[®] In Vivo* Gene Delivery System be used in organisms other than mice?

Although some customers have used *TransIT[®] In Vivo* Gene Delivery System on organisms other than mice, Mirus has tested *TransIT[®] In Vivo* Gene Delivery System only on mice and rats. We recommend increasing the total injection volume to 1/10 of the rat's body weight, using 50-100 ug DNA while maintaining the same ratio of Polymer Solution to DNA(1:1). The maximum total injection volume for the average rat should not exceed 30 ml.

2.3 Can the *TransIT[®] In Vivo* Gene Delivery System be administered via alternate routes?

Some customers have administered *TransIT[®] In Vivo* Gene Delivery System through alternate routes. However, Mirus has tested both tail vein and intramuscular injections. For intramuscular injections, Mirus recommends scaling down the total injection volume to 200 µl while maintaining the same DNA to Polymer Solution ratio, using 10 ug DNA. We recommend formulating in 290 mM Glucose rather than water. If further dilution is required for larger animals, 1X Delivery Solution or 0.9% saline can be used.

2.4 Does the *TransIT[®] In Vivo* Gene Delivery System have any toxic side effects?

Mirus has examined the levels of several enzymes associated with liver toxicity. Two animals were tested for each enzyme at 1 day, 2 days, and 1 week post injection. We examined the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALA), alkaline phosphatase (ALKP) and total bilirubin. Both the AST and ALP enzymes were elevated at 1 day post injection. Both AST and ALP returned to normal by the second day post injection and remained normal through 1 week post injection. The ALKP and bilirubin levels did not change at any time within one week following injection.

2.5 What anesthesia should I use?

Although Mirus no longer uses anesthesia, some researchers may choose to do so. It is preferable to use inhalent anesthetics because Ketamine and other CNS injectable anesthetics have been found, by Mirus and other researchers, to slow the metabolic rate of the mouse down enough to create post-injection recovery problems, resulting in some fatalities. Therefore these reagents should be used with caution.

DISCLAIMER: For research use only.

Small-animal research is regulated by federal laws and regulations. Extensive information on this topic can be found at the NIH Office for Protection from Research Risks (<http://www.nih.gov/grants/oprr/oprr.htm>) and in the "Guide for the Care and Use of Laboratory Animals" from the National Research Council (also available at <http://www.nih.gov/grants/oprr/tutorial>). **This kit does not confer any approval from regulatory agencies to conduct animal research.**

2.6 If I choose not to anesthetize my mouse, how should I restrain it during the procedure?

If a commercial animal restraint is not available, one can be made as described here and in section 3.1 of the protocol. Cut a small opening, approximately 3-5 mm in diameter, in the bottom of a 50 ml conical tube. This opening will facilitate the animal's breathing during the injection procedure. Create a slit opening (~5-8 mm in width) in the cap end of the tube to allow for tail exposure. This opening should only be large enough for the diameter of the tail. When performing injections, put the mouse inside the tube, place the tail through the opening, and screw the cap carefully onto the tube. The tail should now be exposed through the opening.

2.7 What should I use for my negative control?

We recommend using the 1X delivery solution as your negative control. We do NOT recommend using PBS due to the wide variation in recipes found in various reference materials and protocols.

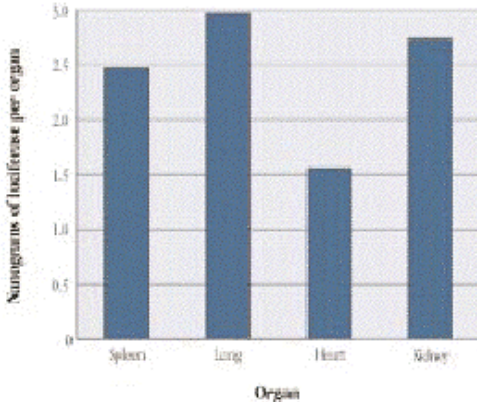
2.8 I have never done tail vein injections before, do you have any suggestions for practicing?

To get a feel for the injection procedure, we suggest injecting an orange with water. This will allow you to get used to holding the syringe with one hand, the orange (substitute mouse) with the other while injecting 3 mL of water in 6-8 seconds. Please note that injecting faster than 6 seconds can be detrimental to the recovery of the animal.

2.9 What purification procedure do you recommend to ensure my nucleic acid is endotoxin free?

We recommend using Mirus' MiraCLEAN™ Endotoxin Removal Kit. It is available in two sizes- MIR 5910 for 10 ug DNA and MIR 5900 for 100 ug DNA.

2.10 What expression level can I expect to get from each of the major internal organs?



Comparison of luciferase gene expression levels in spleen, lung, heart, and kidney tissue following *in vivo* gene delivery. Plasmid DNA (pCI-Luc+) was complexed with the *TransIT*® *In Vivo* Polymer Solution and delivered into ICR (CD-1) mice (Harlan Sprague-Dawley). Twenty-four hours after injection, luciferase expression was measured.

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