*Trans*IT®-EE Hydrodynamic Delivery Solution

Protocol for MIR 5340

SDS and Certificate of Analysis available at mirusbio.com/5340

INTRODUCTION

*Trans*IT®-EE (Enhanced Expression) Hydrodynamic Delivery Solution is designed specifically for the delivery of DNA expression vectors into laboratory mice using the hydrodynamic tail vein injection procedure. This formulation is designed to produce high-level gene expression from the delivered DNA in the liver with significant but reduced levels of expression in the spleen, lungs, heart, and kidneys. This formulation offers the additional benefit that the injected mice demonstrate enhanced expression post-injection compared to animals injected using normal saline.

The expression level obtained using this solution is approximately 2-3 fold greater than the level of expression obtained after delivery of DNA using the *Trans*IT®-QR Hydrodynamic Delivery Solution. The *Trans*IT®-EE Delivery Solution is not optimal for the delivery of siRNA and does not promote the same quick recovery as the *Trans*IT®-QR Delivery Solution.

SPECIFICATIONS

Concentration	Ready-to-use 1X solution.
Storage	Store at 4°C.
Sterility	Filter-sterilized (0.22 µm filter) and tested for endotoxin, RNase and DNase activity.
Product Guarantee	Guaranteed for 1 year from the date of purchase, when properly stored and handled.

MATERIALS

Materials Supplied

*Trans*IT®-EE Hydrodynamic Delivery Solution is supplied in the following format.

Product No.	Quantity
MIR 5340	Sufficient Solution (120 ml) to perform 40 hydrodynamic mouse tail vein injections

Materials Required, but Not Supplied

- Heat source (warm water (37°C) or heat lamp with 120 W bulb)
- Mice of desired strain (18-25 g in weight)
- Nucleic acid (high quality/purity DNA)
- Mouse restraint device
- 3 ml syringes
- 27 gauge needles
- Alcohol swabs

For Research Use Only

DISCLAIMER

For research use only. Small-animal research is regulated by federal laws and regulations. Extensive information on this topic is provided by the NIH Office for Protection from Research Risks (http://www.hhs.gov/ohrp/). This kit does not confer any approval from regulatory agencies to conduct animal research. Follow all applicable laws and regulations pertaining to the care and use of animals in research. All personnel who handle animals should be properly trained. Familiarity with performing tail vein injections in your particular mouse species will greatly facilitate this procedure.

HYDRODYNAMIC TAIL VEIN DELIVERY PROTOCOL

A. Nucleic Acid Preparation

1. Determine the required total injection volume by using the following formula:

   $$\text{Total volume needed per mouse (in ml)} = \frac{\text{mouse weight (g)}}{10} + 0.1 \text{ ml Delivery Solution*}$$

   *The addition of the 0.1 ml of Delivery Solution represents the void volume that remains in the syringe and needle after injection.

   NOTE: Optimal mouse weight is between 18-25 g, which requires 1.9-2.6 ml of injection volume per mouse.

2. Determine the volume of DNA needed for the injection. Mirus Bio recommends 1-50 µg as a starting range. Ten µg is a good starting point, but a titration may be beneficial for optimal delivery.

3. Subtract the volume of DNA from the total injection volume (from Step 1) needed. The remainder represents the volume of *Trans*IT®-EE Hydrodynamic Delivery Solution needed.

   For example, to inject 10 µg of nucleic acid into a 20 g mouse:

DNA stock (1 mg/ml)	10 µl
*Trans*IT®-EE Hydrodynamic Delivery Solution*	2.09 ml
Total Volume	**2.1 ml**

   *An additional 0.1 ml of Delivery Solution is added to accommodate for void volume. The nucleic acid and Delivery Solution can be scaled up for additional mice as needed for replicate injections.

4. Immediately prior to injection, add DNA (from Step 2) to a sterile plastic tube.
5. Add the required volume of Delivery Solution (from Step 3) to the tube containing the nucleic acid and mix well. Inject the DNA/Delivery Solution within 30 minutes of mixing.
6. Connect the needle to the syringe and fill with the entire injection solution, ensuring that no air bubbles are present in the needle or syringe. With the needle pointing up, finger tap the syringe a few times to move air bubbles to the needle and carefully eject the air until a small volume of solution is ejected.

Use sterile technique to prepare nucleic acids for *in vivo* delivery.

Use high-quality DNA that is free of endotoxin and contaminating protein. If necessary, endotoxin can be efficiently removed from DNA using MiraCLEAN® Endotoxin Removal Kit (MIR 5900).

The *Trans*IT®-EE Hydrodynamic Delivery Solution alone can be used as a negative control in parallel injections (recommended).

B. Preparation of Animal for Injection

NOTE: Use of anesthesia is optional. Small doses of inhalant anesthetics work well but require access to a scavenger/fume hood to rid the area of the anesthetic fumes. Follow standard, approved anesthesia practices to reach an induction plane conducive for this technique. Mirus Bio does not recommend the use of injectable anesthesia. Anesthesia is generally not required when a restraint device is used (not provided).

1. To facilitate tail vein visualization and ensure optimal injections, dilate the tail vessels immediately prior to injection by warming the tail of the mouse with a safe, effective heat source (e.g. warm water (~37°C) or heat lamp (120 W bulb)) for 3-5 minutes. As the mouse tail warms up, the vein should dilate and become more visible. Do not overheat the mice with the heat lamp. Excessive movement and/or perspiration are indicators of overheated mice.
2. Use a restraint device to secure the mouse during the injection.

Generally, younger laboratory mice (~5-6 weeks old) are optimal for gene delivery. Mice that are older or have more body fat may exhibit compromised gene delivery. We recommend starting with mice that are 18-25 g each.

C. Injection

1. While working under a light source, locate the dilated vein on the ventral side of the mouse tail, preferably near the distal end (tip) of the tail. Swab the area with an alcohol swab and allow it to air dry to further increase vein visibility and clean the injection site.
2. Place the syringe needle nearly parallel to the tail with the bevel down (toward the tail). Insert the needle into the tail vein. Check needle placement by injecting a small volume in the vein. If the needle is inserted correctly, the vein should begin to clear of blood. If there is a significant resistance, the needle may not be properly inserted into the tail vein. Improper needle insertion into tail tissue is characterized by discoloration and local swelling. If this occurs, remove the needle, and reposition it correctly moving further proximal on the tail.
3. Insert almost the full length of the needle into the vein (to prevent accidental removal of the needle while injecting). Dispense the complete volume of injection solution into the mouse tail vein within 4-7 seconds at a constant rate. A good injection is characterized by a constant resistance that does not increase during the procedure.

Ensure the *Trans*IT®-EE Hydrodynamic Delivery Solution/DNA mix is at room temperature before injecting the mouse. Inject the Delivery Solution/DNA within 30 minutes of mixing.

IMPORTANT: Best results are achieved by a rapid injection at a constant speed, delivering the entire contents of the syringe to the tail vein in 4-7 seconds at a constant rate.

APPLICATION NOTES

Gene Expression Studies

Following gene delivery, animals can be kept for the desired period of time prior to assaying for gene expression. Optimal results are usually obtained 8-24 hours after injection, but this may vary depending on many parameters such as the promoter used to drive transgene transcription, the target organ, and the transgene itself. Therefore, it may be necessary to optimize the conditions (promoter, construct, target organ, kinetics of expression, etc.) for specific applications. For example, when using the CMV promoter, expression tends to decline rapidly after 24 hours; however, lower levels of reporter gene expression may still be detected after 3 weeks.

For secreted proteins, expression can be determined in serum samples obtained at various time points after injection (follow the appropriate protocol for collecting blood). For the analysis of cellular proteins or nucleic acids, the animal may be euthanized (follow the appropriate protocol) at the desired time after injection. Remove the organs (s) of interest and prepare the tissue for assay.

When using immunocompetent mice, the expression of foreign proteins may induce an immune response that could result in the elimination of the cells expressing these proteins.

REFERENCES

1. Zhang, G., et al. (1997) Human Gene Therapy 8:1763-72.
2. Liu, F., et al. (1999) Gene Therapy 6:1258-66.

BIBLIOGRAPHY

1. Methods of Animal Experimentation, Vol. 1 (1965) Gay, W.I., ed., Academic Press, New York.
2. Feldman, D.B., and Seely, J.C. (1988) Necropsy Guide: Rodents and the Rabbit, CRC Press.

TROUBLESHOOTING GUIDE

Problem	Solution
Difficulty with injection	In some mouse strains it is difficult to see the contrast between the tail vein and the tail tissue. Familiarity with tail vein injections in your particular mouse species will minimize injection difficulties. If you have difficulty visualizing the tail vein, warm the mouse tail for 3-5 minutes and swab the tail with an alcohol swab and allow to air dry to help visualize the tail vein.
	Introduce the needle near the tip (distal portion) of the tail. This allows for better observation of the needle entering the vein. If subcutaneous hemorrhaging occurs, the needle can be moved further up the vein (towards the proximal end) to a new injection site.
	If the needle is positioned properly upon injection, clearing of the vein will be apparent and there will be no local swelling or discoloration of the tail. If needed, reposition the needle to a new injection site along the tail.
Little or no gene expression	Verify the sequence of plasmid DNA. Use highly purified, sterile, endotoxin- and contaminant-free DNA.
	Optimize time post-injection for assaying gene expression as it can vary by the gene being expressed, promoter sequence and other features of the experimental design. When using immunocompetent mice, the expression of foreign proteins may induce an immune response that could result in the elimination of the cells expressing these proteins. To assess delivery efficiency of nucleic acids, use Mirus *Label* IT® Kits or prelabeled *Label* IT® Delivery Controls (please refer to Related Products on Page 6).

Call Mirus Bio Technical Support Team at 1.888.530.0801 or email techsupport@mirusbio.com for access to an online instructional video for performing tail vein injections and any specific questions or concerns.

RELATED PRODUCTS

- *Trans*IT®-QR Hydrodynamic Delivery Solution
- MiraCLEAN® Endotoxin Removal Kit
- *Label* IT® Tracker™ Intracellular Nucleic Acid Localization Kits
- *Label* IT® siRNA Tracker™ Intracellular Localization Kits
- *Label* IT® Plasmid Delivery Controls

For details on the above-mentioned products, visit www.mirusbio.com

Contact Mirus Bio for additional information.

Mirus Bio LLC
5602 Research Park Blvd, Ste 210
Madison, WI 53719
Toll-free: 888.530.0801
Direct: 608.441.2852
Fax: 608.441.2849