*Trans*IT®-QR Hydrodynamic Delivery Starter Kit

Product	Quantity	Product No.
*Trans*IT-QR Hydrodynamic Delivery Starter Kit	Sufficient materials to perform 10 hydrodynamic mouse tail vein injections	MIR 5210

Each Starter Kit contains 30 ml of *Trans*IT-QR Hydrodynamic Delivery Solution, a restraint device, 10 syringes, 10 needles and 10 alcohol swabs for hydrodynamic tail vein injections in 10 mice (30 g or less).

1.0 DESCRIPTION

1.1 General Information

The *Trans*IT-QR (Quick Recovery) Hydrodynamic Delivery Solution is designed specifically for the safe and efficient delivery of nucleic acids into laboratory mice using the hydrodynamic tail vein injection procedure. This formulation is optimized for efficient delivery of naked nucleic acids to the liver, with the additional benefit that the injected mice demonstrate quick recovery (QR) post-injection compared to animals injected using normal saline. This Delivery Solution, combined with the hydrodynamic tail vein injection procedure, can be used to deliver either siRNA, for gene knockdown studies, or DNA for gene expression studies in the liver (with significant but reduced levels of delivery to the spleen, lungs, heart, and kidneys.).

The Delivery Solution provided with this kit is certified RNase-, DNase-, and endotoxin-free and provided in a ready-to-use 1X format.

1.2 Materials Supplied

- 30 ml *Trans*IT-QR Hydrodynamic Delivery Solution
- Mouse restraint device
- Ten 3 ml syringes
- Ten 27 gauge needles
- Ten alcohol swabs

1.3 Materials Required but Not Supplied

- Heat source (warm water (37ºC) or heat lamp with 120W bulb)
- Mice of desired strain (18-25 g in weight)
- Nucleic acid (high quality/purity DNA or siRNA)

1.4 Specifications

Concentration: *Trans*IT-QR Hydrodynamic Delivery Solution is a ready-to-use 1X solution.

Storage: Store the *Trans*IT-QR Hydrodynamic Delivery Solution at 4°C. Store all other supplied components at room temperature.

Sterility: *Trans*IT-QR Hydrodynamic Delivery Solution is filter-sterilized (0.22 µm filter) and tested for endotoxin, RNase, and DNase activity.

Stability: The *Trans*IT-QR Hydrodynamic Delivery Solution is stable for 1 year from the date of purchase.

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.

NOTICE: *In vivo* hydrodynamic delivery of non-viral nucleic acids is covered by world wide patents and patent applications of Mirus Bio Corporation, including U.S. Patent 6,627,616; 6,379,966 and related filings worldwide. Purchase of this product does not provide a license to this delivery technology, which is required for all research and commercial uses by for-profit entities. To inquire about a license, please contact Mirus Bio Corporation.

2.0 PROCEDURE

2.1 Nucleic Acid Preparation

NOTE: Use sterile technique to prepare nucleic acids for *in vivo* delivery. Use high-quality DNA ($A_{260}/A_{280} \geq 1.8$) that is free of endotoxin and contaminating protein. If necessary, endotoxin can be efficiently removed from DNA using Mirus Bio's MiraCLEAN® Endotoxin Removal Kit (MIR 5900, 5910). Use siRNA of high quality and of the proper sequence.

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

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

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

For example, a 20 g mouse would require a total volume of 2.1 ml.

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 nucleic acid needed for the injection. Mirus Bio recommends 1-50 µg as a starting range. Ten µg of DNA or 40 µg for siRNA are good starting points, but a titration may be beneficial for optimal delivery.

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

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

Nucleic acid stock (1 mg/ml)	10 µl
*Trans*IT-QR 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 nucleic acid (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 nucleic acid/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.

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

2.2 Injection Protocol

A. Preparation of Animal for Injection

NOTE: 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.

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 the provided restraint device is employed.

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 (120W 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 the provided restraint device to secure the mouse during the injection. The small opening at the bottom of the tube is designed to facilitate the animal's breathing during the injection procedure. The slit opening in the cap end of the tube is designed to allow tail exposure. Place the mouse head first into the tube, gently place the tail through the slit, and then screw the cap carefully onto the tube. The tail should now be easily accessible. For smaller mice, adding paper toweling or cheese cloth to the tube before screwing on the cap may provide more security. The restraint device can be taped to a table to maintain orientation.

B. Injection

NOTE: Ensure the *Trans*IT-QR Hydrodynamic Delivery Solution/nucleic acid mix is at room temperature before injecting the mouse. Inject the Delivery Solution/ nucleic acid within 30 minutes of mixing.

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 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 injection volume 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.

IMPORTANT: Maximum *in vivo* delivery is 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.

2.3 Gene Expression and Knockdown Studies

Following DNA 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.

Following siRNA delivery, animals can be kept for the desired period of time before assaying for target gene knockdown. Mirus Bio scientists normally analyze knockdown efficiency at 24 hours post-injection of the siRNA. However, optimal post-injection times may vary depending on different parameters including the target mRNA, the half-life of the encoded protein, and the siRNA sequence. Careful optimization of post-injection times should be performed to obtain the most consistent and robust knockdowns.

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 organ(s) of interest and prepare the tissue for assay.

NOTE: 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.

3.0 TROUBLESHOOTING

Call Mirus Bio's 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.

Difficulty With Injection

- **Visualizing the tail vein:** 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 (see Section 2.2A). Alternatively, swab the tail with an alcohol swab and allow to air dry to help visualize the tail vein.

- **Site of initial injection:** 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.

- **Position of needle:** 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.

For specific questions or concerns, please contact Mirus Bio Technical Support at 888.530.0801 or techsupport@mirusbio.com
For a list of citations using Mirus Bio products, please visit the Technical Resources section of our website at www.mirusbio.com

4.0 REFERENCES

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

5.0 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.

6.0 RELATED PRODUCTS

***In Vivo* Quick Recovery Delivery Solution:**
*Trans*IT®-QR Hydrodynamic Delivery Solution (Product # MIR 5240)

***In Vivo* DNA Vector Enhanced Expression Delivery Solution and Kit:**
*Trans*IT®-EE Hydrodynamic Delivery Solution (Product # MIR 5340)
*Trans*IT®-EE Hydrodynamic Delivery Starter Kit (Product # MIR 5310)

In Vivo* Gene Delivery Kits with Polymer Solution:
*Trans*IT®-*In Vivo* Gene Delivery System (Product # MIR 5100)

Endotoxin removal from DNA:*
MiraCLEAN® Endotoxin Removal Kit (Product #5900)

DNA tracking studies:
Label IT® Tracker™ Intracellular Nucleic Acid Localization Kit (Product # MIR 7010,7011,7012,7013,7014,7015)

siRNA tracking studies:
Label IT® siRNA Tracker Intracellular Localization Kit (Product # MIR 7212, 7213, 7214, 7215, 7216, 7217)

Determination of gene expression efficiency:
Beta-Gal Staining Kit (Product # MIR 2600)

*These products are available in additional sizes.

The performance of this product is guaranteed for 1 year from the date of purchase if stored and handled properly.
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