I. Introduction

The **Lenti-X™ T-Cell Transduction Sponge** expedites and streamlines lentiviral transduction of human T cells, eliminating the need for prior activation or spinoculation with chemical enhancers. Consisting of a macroporous alginate matrix infused with an optimized blend of rhIL-2 and anti-human CD3 and CD28 antibodies, the Lenti-X T-Cell Transduction Sponge activates human T cells while simultaneously enhancing lentiviral transduction through gentle co-localization of activated T cells and lentivirus particles. This protocol provides instructions for performing lentiviral transduction of human T cells using the Lenti-X T-Cell Transduction Sponge User Manual. The user-friendly protocol minimizes cell manipulation and reduces total reaction volumes while producing transduction efficiencies that are comparable or improved over traditional methods.

II. Required Materials

This protocol applies to the following Takara Bio products:

- Lenti-X T-Cell Transduction Sponge (Cat. No. 631480)

Additional materials required:

- 1X PBS containing Ca^{2+} and Mg^{2+}
- Lentivirus stock of sufficient titer (>1 x 10^7 IFU/ml)
- Appropriate complete cell culture medium including desired cytokines for T cells
- Tissue culture incubator (5% CO_2, humidified)
- Non-treated tissue culture plate, 24-well plate
- 15 ml conical tubes
- 10 mL filtered pipets and pipetting device
- Micropipettes and tips (with hydrophobic filters)
- Centrifuge
- Scissors

III. Protocol Overview

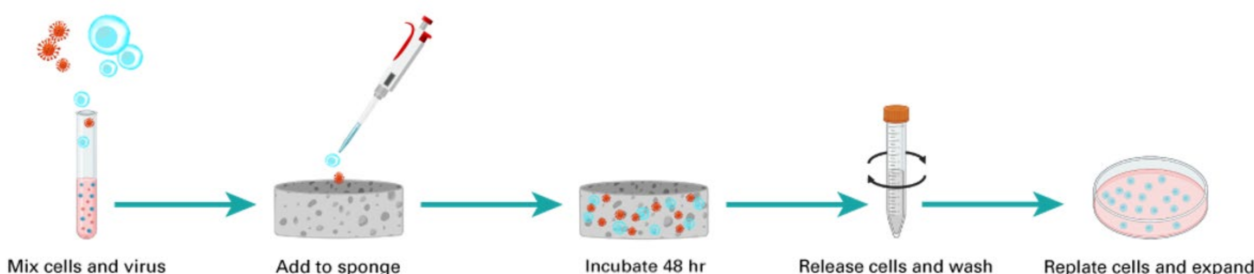

Figure 1. Lenti-X T-Cell Transduction Sponge workflow.

IV. Safety Guidelines

Pseudotyped lentivirus packaged from HIV-1-based vectors are capable of infecting human cells and work involving lentiviral vectors requires the use of a Biosafety Level 2 facility. For your safety and the safety of others around you, it is imperative to fully understand the potential hazards of working with recombinant lentiviruses and the necessary precautions for their use in the laboratory.

For more information on Biosafety Level 2 agents and practices, download the following reference: CDC & NIH. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition | CDC Laboratory Portal | CDC. U.S. Dep. Heal. Hum. Serv. (2024) at https://www.cdc.gov/labs/BMBL.html.

V. Protocol

In the Lenti-X T-Cell Transduction Sponge protocol, human T cells and lentivirus are first incubated together within the sponge to promote activation and transduction. Then, transduced target cells are released from the sponge through depolymerization of the alginate, washed, and plated for downstream use. This protocol can be used as a starting point for determining the optimal conditions for your T cells and is for the application of one sponge.

A. Before you Start

- For best results, culture T cells in a manner that will ensure high viability and log-phase growth, if possible.
- Adjust unstimulated cell concentration to ~5 x 10^7 to 1 x 10^8 cells/ml in the appropriate tissue culture media. This allows for proper cell numbers while adjusting for virus volume to a total of 150 µl.
- Thaw lentiviral stock on ice until ready to use.

  NOTE: We recommend a starting lentiviral titer of 1 x 10^7 IFU/ml to allow a reasonable multiplicity of infection (MOI) to be applied during transduction while also minimizing the total transduction volume. If the lentiviral titer is low or unknown, we recommend determining the titer using Lenti-X GoStix™ Plus (Takara Bio, Cat. No. 631280) and concentrating the lentivirus to reduce volume and increase the titer that is applied to the Lenti-X T-Cell Transduction Sponge. Lenti-X Concentrator (Takara Bio, Cat. No. 631231) can be used for concentration of lentiviral stocks.

B. Day 1

IMPORTANT: All steps in this protocol should be performed in a sterile tissue culture hood.

1. Create a transduction mix consisting of target cells and lentivirus at the desired MOI. Total transduction volume (inducing target cells, virus, and media) should not exceed 150 µl.

   NOTE: For a single sponge, we recommend using a range of 2 x 10^6 to 1 x 10^7 target cells and an MOI range of 1–10.

2. Using scissors, cut out the desired number of Lenti-X T-Cell Transduction Sponges from the packaging, making sure to cut between the sponges without disturbing adjacent well seals. Return the remaining sponges to the foil pouch and reseal.
3. Peel off the foil covering the sponge and use forceps (provided) to transfer a sponge to a single well of a sterile, 24-well non-treated tissue culture plate. Allow the sponge to equilibrate to room temperature for 2–3 min.

   NOTES:

   – It is important to peel off the foil covering to remove the sponge. Do not "pop" sponges out of the packaging through the foil as this could damage the sponge structure.

Figure 2. Transferring the sponge to a single well of a sterile, 24-well non-treated tissue culture plate.

4. Slowly add the entire volume of the transduction mix across the top of the sponge in a dropwise manner, ensuring that the mixture remains on the top of the sponge and does not spill down the sides. The entire volume will be absorbed slowly into the sponge.

   NOTE: If the maximum volume of 150 µl is exceeded, the transduction mixture may spill or leak, and activation/transduction efficiency may be reduced. If spillage of the transduction mixture does occur, simply pipette the spilled portion back onto the sponge. Spillage can also be avoided by ensuring that there is no contact between the sponge and the side of the well.

5. Incubate the plate at 37ºC in a tissue culture incubator for 1 hr. The sponge will continue to absorb the transduction mixture.

Figure 3. Absorption of the transduction mixture into the sponge after 1 hr.

6. After 1 hr, add 1 ml of complete cell culture medium + IL-2 (or preferred cytokine combination) to the well containing the sponge, taking care to not pipette directly on the sponge.
7. Incubate at 37ºC for 48hr.

   NOTE: Extended culture beyond the recommended 48 hr in the sponge is not recommended and can lead to reduced cell viability.

C. Day 2

1. Transfer the media from the well containing the sponge and place it into a 15 ml conical tube.

   CAUTION: Supernatant contains infectious lentivirus.

2. Using aseptic technique, use a 20 µl pipette tip to pierce and pick up the sponge and transfer it to the 15 ml tube containing the media from Step 1 (Figure 5).

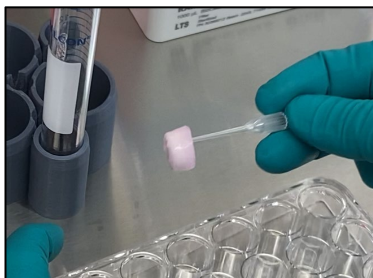

Figure 4. Transferring the sponge to a 15 ml tube using a 20 µl pipette tip.

NOTE: The sponge may not settle to the bottom of the tube. This does not affect the efficiency of release or cell viability. Do not attempt to force the sponge to bottom of tube.

3. Add 1 ml of Release Buffer to the 15 ml tube.
4. Centrifuge at 1,000*g* for 2 min at room temperature to dissolve the sponge and release the cells. Do not remove the supernatant.

   NOTE: The sponge may not appear to completely dissolve during this centrifugation step. This is expected, and the sponge will completely dissolve in the subsequent washing steps.

5. Add 10 ml 1X PBS (with Ca^{2+}/Mg^{2+}) and invert the tube gently five times to mix. Do not vortex.
6. Centrifuge the sample at 500*g* for 10 min and discard the supernatant. Take care not to disturb the cell pellet.
7. Repeat Steps 5 and 6. Be sure to remove the remaining supernatant from pellet.
8. Resuspend the cell pellet in a complete culture media and transfer to the desired culture vessel.
9. Incubate at 37°C (5% CO_2; humidified) and continue with your desired application.

For instructions on troubleshooting this protocol, refer to the Lenti-X T-Cell Transduction Sponge User Manual.

Contact Us	
Customer Service/Ordering	**Technical Support**
tel: 800.662.2566 (toll-free)	tel: 800.662.2566 (toll-free)
fax: 800.424.1350 (toll-free)	fax: 800.424.1350 (toll-free)
web: takarabio.com/service	web: takarabio.com/support
e-mail: ordersUS@takarabio.com	e-mail: technical_support@takarabio.com

Notice to Purchaser

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without our prior written approval.

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product's web page at takarabio.com. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.

© 2025 Takara Bio Inc. All Rights Reserved.

All trademarks are the property of Takara Bio Inc. or its affiliate(s) in the U.S. and/or other countries or their respective owners. Certain trademarks may not be registered in all jurisdictions. Additional product, intellectual property, and restricted use information is available at takarabio.com.

This document has been reviewed and approved by the Quality Department.