Takara Bio USA

Lenti-X™ T-Cell Transduction Sponge User Manual

Cat. No. 631480
(050525)

Takara Bio USA, Inc.
2560 Orchard Parkway, San Jose, CA 95131, USA
U.S. Technical Support: technical_support@takarabio.com

United States/Canada	Asia Pacific	Europe	Japan
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.565.6999

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I. Introduction

The **Lenti-X T-Cell Transduction Sponge** (Cat. No 631480) expedites and streamlines lentiviral transduction of T-cells, bypassing the need for prior activation or spinoculation with chemical enhancers. This innovative approach employs a macroporous alginate matrix infused with an optimized blend of rhIL-2 (100 IU), anti-human CD3 antibody, and anti-human CD28antibody. This combination within the sponge not only activates T-cells but also enhances lentiviral transduction by fostering the co-localization of activated T-cells and lentivirus particles. The straightforward protocol minimizes cell manipulation and reduces reaction volumes while yielding transduction efficiencies on par with or superior to traditional techniques.

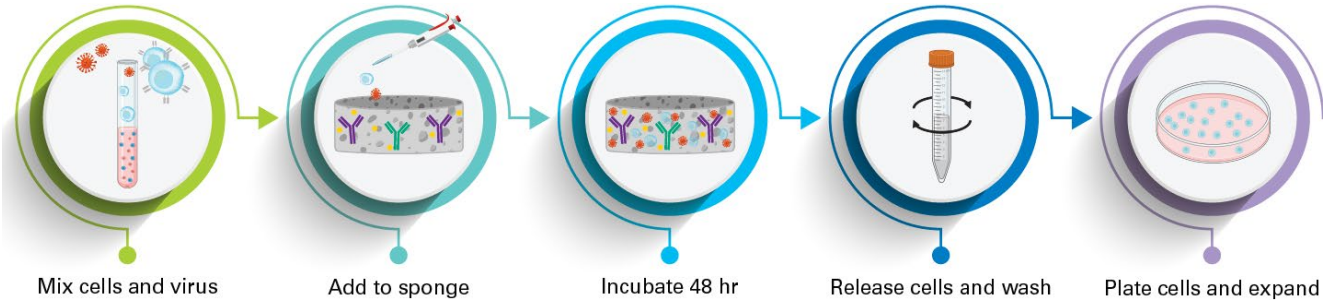

Figure 1. Lenti-X T-Cell Transduction Sponge workflow.

II. List of Components

Lenti-X T-Cell Transduction Sponge (Cat. No. 631480) contains sufficient items for twelve transduction reactions.

Table 1. Lenti-X T-Cell Transduction Sponge components.

Lenti-X T-Cell Transduction Sponge (Store at 4°C)	631480 (12 rxns)
Lenti-X T-Cell Transduction Sponge*	12 rxns
Release Buffer	30 ml
Forceps	1 each

*Store Lenti-X T-Cell Transduction Sponges in the supplied foil pouch containing the desiccant sachet. Return unused sponges to the supplied foil pouch when storing.

III. Additional Materials Required (Not Provided)

- 1X PBS containing Ca^{2+} and Mg^{2+}
- Lentivirus stock of sufficient titer (>1 x 10^7 IFU/ml)
- Complete cell culture medium for T-cells (e.g., LymphoONE® T-Cell Expansion Xeno-Free Medium [Cat. No. WK552S or WK552] or an appropriate equivalent)
- Tissue culture incubator (5% CO_2, humidified)
- Non-treated tissue culture plate, 24-well (e.g., Falcon 24-well Polystyrene Clear Flat Bottom Not Treated Cell Culture Plate; Corning, Inc., Prod. No. 351147)

  IMPORTANT: Treated tissue culture plates are commonly charged, which can make placement of the sponge in the center of the well challenging. Please ensure you use non-treated plates.

- 15 ml conical tubes
- 10 ml filtered pipettes and pipetting device
- Micropipettes and tips (with hydrophobic filters)

- Centrifuge
- Scissors

Optional materials:

- **Day 1 (Section V.B)**
  - Lenti-X Concentrator (100 ml) (Cat. No. 631231)
  - Lenti-X GoStix™ Plus (20 Tests) (Cat. No. 631280)
  - Lenti-X p24 Rapid Titer Kit (Single Wash) (Cat. No. 631476)
- **Troubleshooting (Appendix)**
  - Lenti-X Concentrator (100 ml) (Cat. No. 631231)
  - Lenti-X Packaging Single Shots (VSV-G) (Takara Bio, Cat. No. 631275)
  - Lenti-X Purification Kit (2 preps) (Takara Bio, Cat. No. 631233)

IV. Safety Guidelines

Pseudotyped lentivirus packaged from HIV-1-based vectors are capable of infecting human cells. 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. (2020) at https://www.cdc.gov/labs/BMBL.html.

V. Protocol

In the Lenti-X T-Cell Transduction Sponge protocol, 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 is for the application of one sponge for transducing 2 x 10^6 ~ 1 x 10^7 T-cells or PBMCs.

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.

  Please note that cell counts applied in standard T-cell transduction protocols typically refer to the number of activated cells, by which point some cells have already been lost from the initial unstimulated population.

  However, since the T-cell sponge workflow utilizes unstimulated cells to combine cell activation and transduction into a single step, it may lead to misinterpretation of cell yields and viability when compared directly with standard transduction methods.
- Thaw lentiviral stock on ice until ready to use.
- Prepare appropriate complete cell culture medium for T-cells (including desired cytokines) and pre-warm the medium until use.

B. Day 1

IMPORTANT: All steps in this protocol should be performed in an appropriate biosafety cabinet.

1. Create a transduction mix consisting of target cells and lentivirus at the desired MOI, using the guidelines in Table 2. Total transduction volume (inducing target cells, virus, and media) should not be >150 µl.

Table 2. Transduction mix guidelines.

Component	Parameters
Target cells	Each sponge can accommodate from 2 x 10^6 to 1 x 10^7 cells.
Virus amount	Start with an MOI from 1 to 10 and adjust to meet desired efficiency.
Cell culture medium + cytokine mix	We recommend LymphoONE T-Cell Expansion Xeno-Free Medium (Cat. No. WK552S or WK552), supplemented with IL-2. If alternate medium/cytokines (IL-7, IL-15, etc.) are desired, add them to the medium mix to be loaded onto the sponge.

NOTE: We recommend a starting lentiviral titer of 1 x 10^7 IFU/ml to allow a reasonable 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 (Cat. No 631280) or Lenti-X p24 Rapid Titer Kit (Single Wash; Cat. No. 631476) and concentrating the lentivirus with Lenti-X Concentrator (Cat. No 631231) with a short, 15 min protocol, to reduce volume and increase the titer that is applied to the Lenti-X Transduction Sponge.

2. Using scissors, cut out the desired number of sponges from the packaging, making sure to cut between them without disturbing adjacent well seals (Figure 2). Return the remaining sponges to the foil pouch and reseal.

NOTE: The blister packaging is designed to protect the sponge from compression; resistance to cutting may be noticed at this step.

Figure 2. Proper cutting of the packaging to remove Lenti-X T-Cell Transduction Sponges. Cut between the wells of the blister package. Take care not to disturb the seal on adjacent wells.

3. After peeling back the foil (Figure 3, Panel A), use forceps to transfer a sponge to a single well of a sterile, non-treated 24-well tissue culture plate (Figure 3, Panel B). Allow the sponge to equilibrate to room temperature for 2–3 min.

Figure 3. Transferring the Lenti-X T-Cell Transduction Sponge from the blister pack to the 24-well plate. **Panel A.** Peeling the foil back to extract the sponge from the packaging. **Panel B.** Sponge placement into the well using the forceps provided with the kit.

NOTES:

– Do NOT try to push the sponges out of the package through the foil. Doing so will collapse the sponge's microfluidic channels.

– The forceps provided with the kit, if maintained in sterile condition, can be reused for future experiments. Alternatively, sterile stainless-steel forceps can be used.

4. Slowly add the entire volume of the transduction mix across the top of the sponge in a dropwise manner (Figure 4), ensuring that the mixture remains on the top of the sponge and does not spill down the sides (Figure 5). The entire volume will be absorbed slowly into the sponge; we recommend allowing the mixture to absorb into the sponge to avoid spillage when transferring to the incubator.

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

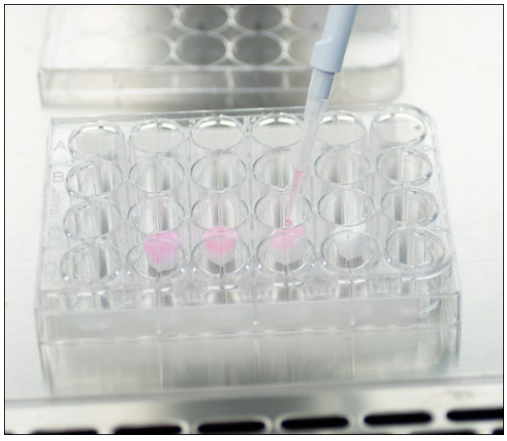

Figure 4. Addition of sample to the sponge

Figure 5. Proper placement of the sponge and sample in a well. The sponge is not touching walls, and the transduction mixture is all on the sponge.

5. Place the lid back on the 24-well plate and incubate the plate at 37ºC for 1 hr in a cell culture incubator. During incubation, the sponge will continue to absorb the transduction mixture (Figure 6).

Figure 6. Post-sample absorption of the sponge

6. After 1 hr, add 1 ml of complete cell culture medium + cytokine mix (see Table 2) along the sides of the well containing the sponge (Figure 7), taking care to not pipette directly on the sponge.

Figure 7. Addition of culture media into a well

7. Incubate at 37ºC for 48 hr to foster activation and transduction processes.

NOTE: Extended culture beyond the recommended 48 hr in the sponge does not yield higher transduction efficiency and is not recommended. Excessive incubation time can lead to reduced cell viability.

C. Day 3

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, pierce and pick up the sponge using a 20 µl pipette tip and transfer it to the 15 ml tube containing the media from Step 1 (Figure 8).

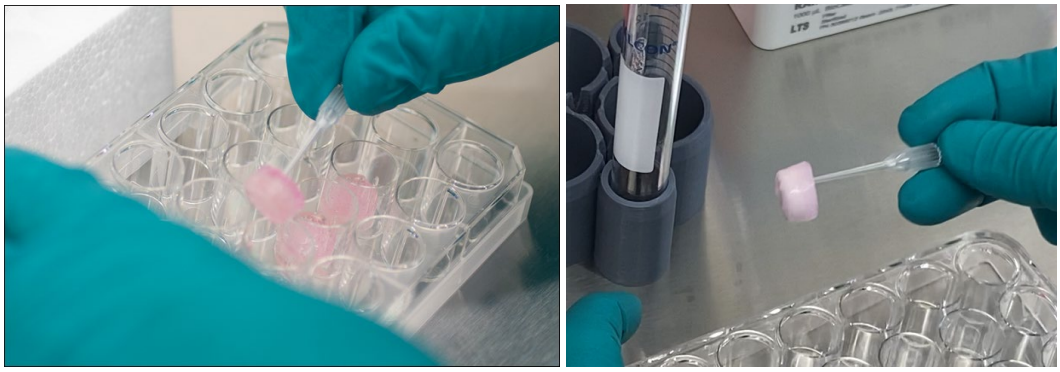

Figure 8. Post-transduction transfer of the sponge using a pipette tip.

3. Add 1 ml of Release Buffer to the 15 ml tube.

NOTE: The sponge may not settle to the bottom of the tube (Figure 9). It will relocate to the bottom during centrifugation in the next step. Do not attempt to force the sponge to the bottom of the tube.

Figure 9. A 15 ml tube containing culture media, the sponge (arrow), and Release Buffer before centrifugation.

4. Centrifuge the tube at 1,000*g* for 2 min at room temperature to facilitate entry of the Release Buffer into the sponge and release the cells (Figure 10). Do not remove the supernatant.

NOTE: The sponge will not completely dissolve during this centrifugation step (Figure 11). This is expected and does not affect the efficiency of release or cell viability. The sponge will completely dissolve after completing the subsequent washing steps.

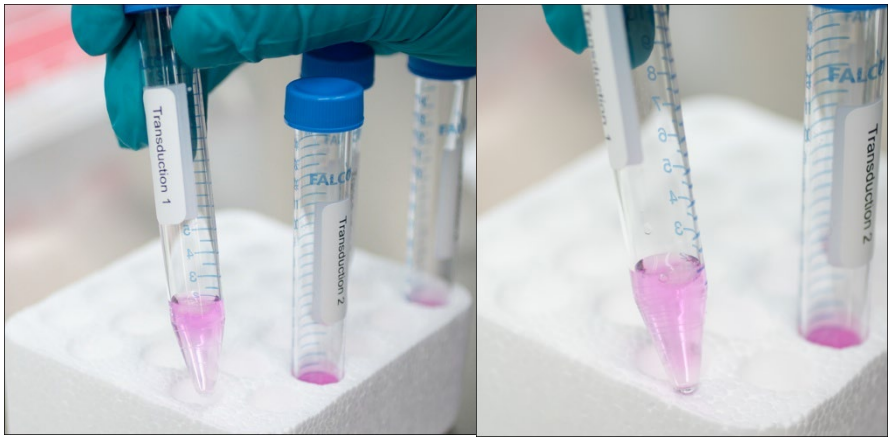

Figure 10. A 15 ml tube containing culture media, the sponge, and Release Buffer after centrifugation. The two images illustrate the sponge submerged in the solution after adding the Release Buffer and centrifugation.

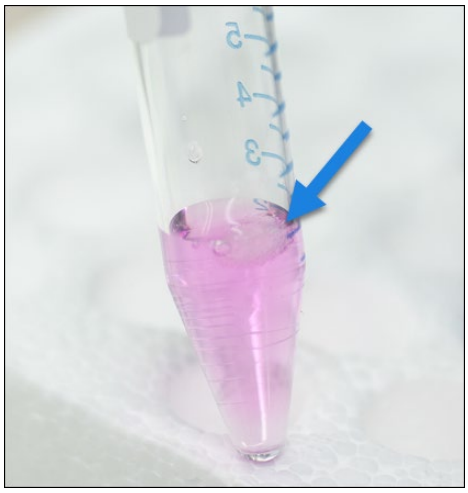

Figure 11. Close-up of the 15 ml tube containing culture media, the sponge, and Release Buffer after centrifugation. A partial sponge may be visible (arrow) .

5. Add 14 ml prewarmed 1X PBS (with Ca^{2+}/Mg^{2+}) (Figure 12).

Figure 12. The conical tube contents after adding 1X PBS.

6. Mix gently five times by inversion (Figure 13). Do not vortex.

Figure 13. Gentle mixing by inversion. Invert and return upright a total of five times.

NOTE: The sponge may not appear to completely dissolve during this inversion step (Figure 14). This is expected, and the sponge will completely dissolve after completing the washing steps. **The residual sponge does not affect cell yields or viability and can be removed without issue.**

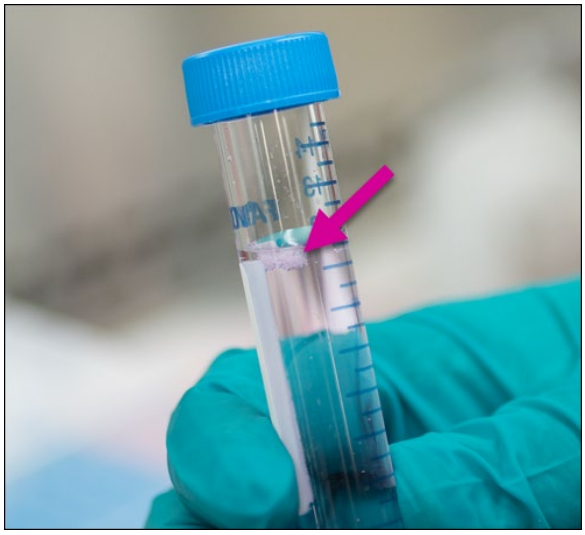

Figure 14. The conical tube and solution after inversion. A partial sponge may still be visible (an arrow) but will not impact the final cell yield or viability. It can be discarded with the wash at this point.

7. Centrifuge the sample at 500*g* for 10 min to collect the cells and discard the supernatant. Take care not to disturb the cell pellet.
8. Repeat Steps 5–7. Be sure to remove the remaining wash solution from the cell pellet.
9. Resuspend the cell pellet in 2 ml of prewarmed complete culture media, count the cells, and plate at the desired cell concentration.

NOTE: Since the T-cell sponge workflow utilizes unstimulated cells to combine cell activation and transduction into a single step, it may lead to misinterpretation of cell yields and viability when compared directly with standard transduction methods.

10. Incubate at 37°C (5% CO_2; humidified) and continue with your desired application.

Appendix. Troubleshooting Guide

Table 3. Troubleshooting guide. Continued on next page.

Problem	Possible Explanation	Solution
The lentivirus titer is lower than 1 x 10^7 IFU/ml.	Lentivirus packaging protocol may not be optimal.	Concentrate the lentivirus with a tool such as Lenti-X Concentrator (Cat. No. 631231)
		Produce high-titer lentivirus using Lenti-X Single Shots (Cat. No. 631275).
Sponge remains visible after completing the wash step	Incomplete dissolution by the release buffer.*	Ensure complete mixing during each of the wash steps.
		Remove the residual sponge† before discarding the remaining PBS solution after the final wash spin in Step 8.
Low transduction efficiency	Insufficient MOI was applied due to titer or sponge volume requirements	Concentrate the lentivirus with a tool such as Lenti-X Concentrator (Cat. No. 631231)
		Produce high-titer lentivirus using Lenti-X Single Shots (Cat. No. 631275).
	Insufficient contact between cells and lentivirus within the sponge.	Enhance crowding of cells and virus in the sponge by increasing cell density (up to 10^7 cells) while maintaining MOI.
	Inadequate incubation time	Incubate the sponge with transduction mixture (cells and lentivirus) for 48 hrs to allow sufficient time for both activation and transduction.
	Low cell viability	Ensure viability is >90% at the time of transduction.
	Viral supernatant contains transduction inhibitors.	Purify your virus prior to transduction using the Lenti-X Maxi Purification Kit (Cat. Nos. 631233 or 631234).

*At the end of the wash steps, all pores of the sponge have been opened, and the cells have been released. Therefore, the presence of residual sponge does not indicate low cell release.

†Removal of the residual sponge does not affect cell yields.

Problem	Possible Explanation	Solution
Low cell-yield post transduction	Standard transduction protocols typically refer to the number of activated cells, which doesn't account for cells lost from activation. The sponge workflow utilizes unstimulated cells to combine cell activation and transduction into a single step, possibly leading to misinterpretation of cell counts depending upon timing of cell counts.	Be sure to account for cell loss during the activation step when comparing the cell counts using the sponge to the standard methods, such as spinoculation with polybrene. Sponge-transduced cell growth rates are comparable to cells transduced by standard approaches.
	Use of prestimulated cells. Because the sponge contains activation reagents (anti-CD3/CD28 antibodies), additional stimulation can induce programmed cell death after repeated activation.	Ensure that cells are unstimulated prior to adding them to the sponge.
T-cell expansion appears to be lower compared to cells transduced using other common methods, such as polybrene or RetroNectin® reagent.	Expansion of sponge-transduced cells is slowed during the 48 hr incubation period in the sponge.	Cells will quickly resume growth once released from the sponge Extend the cell expansion period by a few days to allow the cells to reach the desired yield.

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