# I. Introduction

The **Lenti-X™ Transduction Sponge** (Cat. No. 631478) facilitates rapid and efficient lentiviral transduction without spinoculation or the use of chemical transduction enhancers. Consisting of a macroporous alginate that encourages the colocalization of target cells and lentivirus, the Lenti-X Transduction Sponge eliminates biotransport issues associated with standard lentiviral transduction methods. A simple, easy-to-use protocol reduces cell handling and requires smaller 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 Transduction Sponge (Cat. No. 631478)
  - 24 Lenti-X Transduction Sponges
  - 30 ml Release Buffer
  - 1 Forceps

**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 for target cells
- Tissue culture incubator (5% $CO_2$, humidified)
- Non-tissue culture-treated 24-well plate
- 15 ml conical tubes
- 10 ml filtered pipettes and pipetting device
- Micropipettes and tips (with hydrophobic filters)
- 20 µl pipette tips
- Centrifuge
- Scissors

# III. Protocol Overview

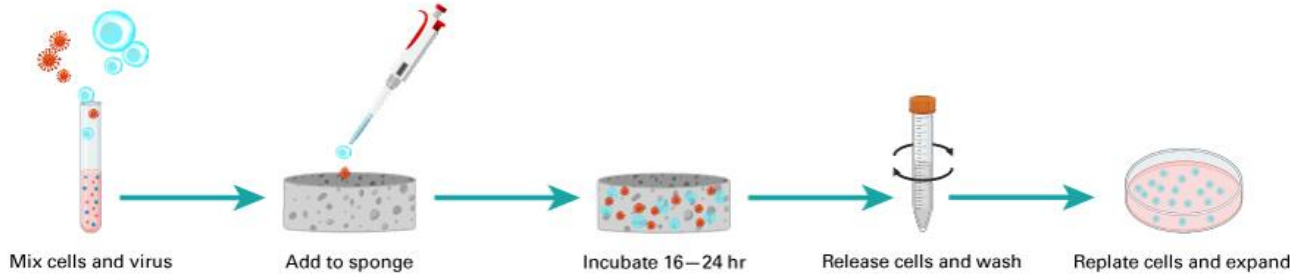


**Figure 1. Lenti-X Transduction Sponge workflow.**

# 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. (2020) at https://www.cdc.gov/labs/BMBL.html.

# V. Protocol

Target cells and lentivirus are first incubated together within the sponge to promote transduction. Then, the transduced target cells are released from the sponge through depolymerization of the alginate, washed, and plated for downstream use. This protocol should be used as a starting point for determining the optimal conditions for your target cells.

## A. Before you Start

- For best results, culture target cells in a manner that will ensure high viability and log-phase growth, if possible
- Adjust cell concentration to 1 x $10^7$ cells/ml in the appropriate tissue-culture media
- 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 concentrating the lentivirus to reduce volume and increase the titer that is applied to the 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 1 x $10^6$ target cells and an MOI within a range of 1–10.
2. Using scissors, cut out the desired number of Lenti-X Transduction Sponges from the packaging, making sure to cut between the sponges without disturbing adjacent well seals, as demonstrated in Figure 2. Return remaining sponges to the foil pouch and reseal.

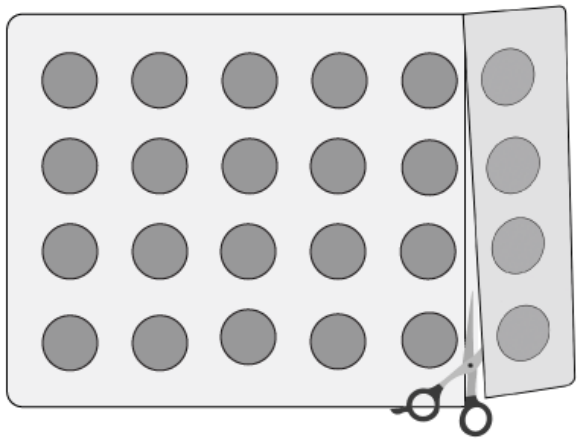

**Figure 2. Proper cutting of the packaging to remove Lenti-X Transduction Sponges.** Cut between the wells of the blister package. Take care not to disturb the seal on adjacent wells.

3. Peel off the foil covering the sponge and use forceps (provided) to transfer a sponge to a single well of a sterile, non-tissue culture treated 24-well plate (Figure 3). 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.
- 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.

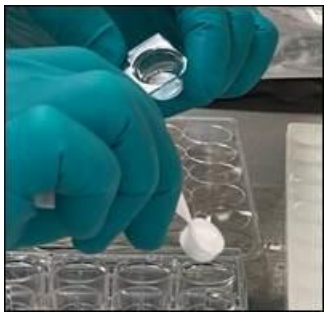

**Figure 3. Transferring the sponge to a single well of a sterile, non-tissue culture treated 24-well plate.**

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 into the sponge.

**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 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.

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

**Figure 4. Absorption of the transduction mixture into the sponge after 1 hr.**

6. After 1 hr, add 1 ml of complete-cell-culture medium to the well containing the sponge, taking care to not pipette directly on the sponge.
7. Incubate at 37ºC overnight (16–24 hr).

**NOTE:** Extended culture beyond 24 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 and a 20 µl pipette tip, pierce and pick up the sponge and transfer it to the 15 ml tube containing the media from Step 1 (Figure 5).

   **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.

**Figure 5. Transferring the sponge to a 15 ml tube using a 20 µl pipette tip.**

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; the sponge will completely dissolve in the subsequent washing steps.
   Bubbles may also appear as air is released from the sponge as it dissolves. These will dissipate in subsequent washes.

5. Add 10 ml 1X PBS (with $Ca^{2+}$/$Mg^{2+}$) and mix gently five times by inversion. 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.
8. Resuspend the cell pellet in a complete-culture media appropriate for your target cell type and transfer to the desired culture vessel.
9. Incubate at 37°C (5% $CO_2$; humidified) and continue with your desired application.

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