TOOLSilent LentiBooster

Cat. no. VBT-KA00

Size: 1 mL

Storage: Store at 4°C.

Introduction

TOOLSilent LentiBooster (500X) is a novel cocktail of small molecules that can enhance viral production and is a powerful, broadly applicable reagent for effective virus packaging. TOOLSilent LentiBooster stably regulates viral RNA packaging at the transcriptional level, which can enhance the production of either retroviral or lentiviral particles up to 10-fold. The easy-to-use protocol makes TOOLSilent LentiBooster well-suited to various scales of virus packaging.

Protocol

1. Day 1: The day before transfection, coat plates/dishes with 1X gelatin for 30 minutes. Aspirate the gelatin, and plate approximately 3–4 × 10^6 HEK 293T cells per 100-mm plate. Use 10 mL of medium for each plate.

Note: It is critical to have good single-cell suspensions (trypsinized completely) and to evenly distribute the cells.

Day 2: Transfection

Note: Prepare your transfection following the manufacturer's protocol.

2. Prepare two tubes and add 0.5 mL of Dulbecco's modified Eagle's medium (DMEM) to each. To one tube, add DNA mixture (containing viral vector and packaging mix) and mix well by tapping the tube. To the other tube, add TOOLSmoothFect Transfection Reagent (Cat. NFT-KA00) and mix by tapping the tube.

Note: Incubate at room temperature (20–25°C) for no longer than 5 minutes.

3. Transfer the TOOLSmoothFect–DMEM mixture into the DNA tube and pipette up and down 2–3 times. Mix well by vortexing for 5–10 seconds.
4. Incubate for approximately 15 minutes at room temperature to allow TOOLSmoothFect/DNA complexes to self-assemble.
5. Add the TOOLSmoothFect/DNA mixture drop-wise onto the plate; gently rock the plate and place it back in the incubator.

Day 3: Change the medium and add TOOLSilent LentiBooster.

6. Replace the supernatant with 10 mL of fresh media and supplement with 20 µL of TOOLSilent LentiBooster (500X). Return the plates to the cell culture incubator.

Day 4: Collect the virus.

CAUTION: Handle virus material with caution and avoid spills. Use bleach to decontaminate hazardous liquids (10% final concentration for 30 minutes).

7. Collect the supernatant in a 50-mL conical tube and put it on ice. Centrifuge the supernatant at 1,000 × *g* for 10 minutes to remove cell debris (with the centrifuge preset to 4°C).
8. Filter the supernatant through a 0.45-µm filter. Transfer the filtered supernatant to a sterile vessel and add 1 volume of cold TOOLSilent LentiPrecipitating Reagent (4°C, Cat no. VCT-KA00) to every 4 volumes of virus-containing supernatant (for example: 5 mL of TOOLSilent LentiPrecipitating Reagent with 20 mL of viral supernatant).
9. Mix well and refrigerate overnight.

Day 5: Concentrate the virus.

10. Centrifuge the mixture at 1,500 × *g* for 30 minutes at 4°C. After centrifugation, viral particles may appear as a beige or white pellet at the bottom of the vessel.
11. Discard the supernatant. Spin down the residual solution by centrifugation at 1,500 × *g* for 5 minutes. Remove all traces of fluid through aspiration, taking great care not to disturb the precipitated viral particles in the pellet.
12. Resuspend the viral pellets in 1/10 to 1/100 of the original volume using cold, sterile phosphate-buffered saline or DMEM at 4°C. Aliquot in cryogenic vials and store at −80°C until ready for use.

Note: TOOLSilent LentiBooster can be removed using viral concentration/purification procedures. The side effect of crude viral particles with TOOLSilent LentiBooster on the expression of the gene of interest has not been detected when it is used directly to transduce HEK 293T cells, but it may vary from cell line to cell line. It is recommended to test the effect of TOOLSilent LentiBooster on the target cells beforehand.

The product is for research only; not for diagnostic or clinical use.