ViralBoost Reagent Protocol

Catalogue number: VB100 ; Size: 1 ml

Description:

ViralBoost Reagent (500X) is a novel cocktail of small molecules that is used for high titer virus production and is a powerful, broadly applicable reagent for effective virus packaging. It stably regulates the viral RNA packaging on the transcriptional level, which can greatly enhance production of either retro- or lenti-viral particles up to 10 fold. The easy-to-use protocol makes ViralBoost Reagent well-suited for various scales of virus packaging.

Protocol:

Day 1: The day before transfection,

1. Coat plates/dishes with 1x Gelatin for 30 min. Aspirate gelatin, and plate ~3-4 X 10^6 HEK 293T cells per 100-mm plate. Use 10 ml medium for each plate.

Note: It is very important to have good single cells suspensions (trypsinize well) and to evenly distribute the cells.

Day 2: Transfection

Note: Prepare your transfection following manufacturer's protocol.

2. Prepare two tubes, and add 0.5 ml DMEM to each tube. To one tube, add DNA mix (containing viral vector and packaging mix) and mix well by tabbing the tube. To the other tube, add NanoFect (Cat. no. NF100). Mix by tabbing the tube.

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

3. Transfer NanoFect-DMEM mixture into the DNA tube, pipette up and down for 2–3 times. Mix well by vortexing for 5-10 seconds.

4. Incubate for ~15 minutes at room temperature to allow for NanoFect/DNA complexes self-assembly.

5. Add the NanoFect/DNA mix drop-wise to the plate, gently rock the plate and place the plate back to the incubator

Day 3: Change medium and add ViralBoost

6. Replace supernatant with 10 ml fresh media and supplement with 20 µl of ViralBoost (500X). Return the plates to the cell culture incubator.

Day 4: Collect virus

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

7. Collect the supernatant in a 50 ml conical tube and put in on ice. Centrifuge the supernatant at 1,000g for 10 min to remove cell debris. (Preset the centrifuge to 4 °C)

8. Filter the supernatant through 0.45 µm filter. Transfer filtered supernatant to a sterile vessel and add 1 volume of cold Retrovirus/Lentivirus Precipitation Solution (4°C, Cat no. VC100 & VC200) to every 4 volumes of virus-containing supernatant. (For example: 5 ml Retrovirus/Lentivirus Precipitation Solution with 20 ml viral supernatant).

9. Mix well and refrigerate overnight.

Day 5: Concentrate virus

10. Centrifuge mixture at 1500g 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 supernatant. Spin down residual solution by centrifugation at 1500g for 5 minutes. Remove all traces of fluid by aspiration, taking great care not to disturb the precipitated viral particles in pellet.

12. Resuspend viral pellets in 1/10 to 1/100 of original volume using cold, sterile PBS or DMEM at 4°C. Aliquot in cryogenic vials and store at -80°C until ready for use.

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

2600 Hilltop Drive, B/C 328, Richmond, CA 94806
www.alstembio.com Tel: (510) 708-0096 Email: info@alstembio.com