Product Specification Sheet

Product Name	**ViralBoost™ Reagent**
Description	ViralBoost™ Reagent (500X) is a novel cocktail of small molecules that can enhance viral production and is a powerful, broadly applicable reagent for effective virus packaging. The ViralBoost™ Reagent stably regulates the viral RNA packaging at the transcriptional level, which can enhance production of either retro- or lentiviral particles up to 10-fold. The easy-to-use protocol makes ViralBoost™ well-suited for various scales of virus packaging.
Catalog Number	VB100
Size	1 ml
Shipping	Ambient Temperature
Storage and Stability	Store at 4° C. This product is stable for 12 months when stored as directed.
Usage	6-14 hours after transfection of human embryonic kidney (HEK) 293T cells with retroviral or lentiviral packaging plasmid mix, replace the culture medium with fresh DMEM medium supplemented with 10% heat-inactivated fetal bovine serum and 0.5% penicillin-streptomycin, and add 1/500 volume of ViralBoost™ Reagent to one volume of fresh culture medium and continue incubation in the CO_2 incubator at 37° C.
Quality Control	Each lot of ViralBoost™ Reagent is functionally tested in transfection assays using human embryonic kidney 293T cells.
Restricted Use	For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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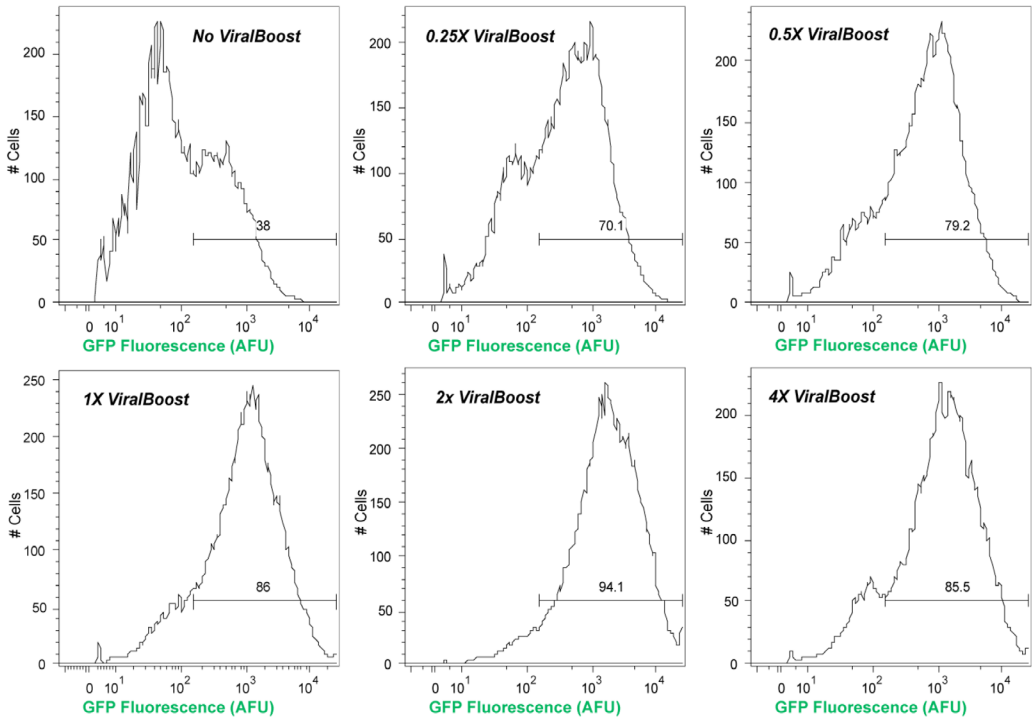
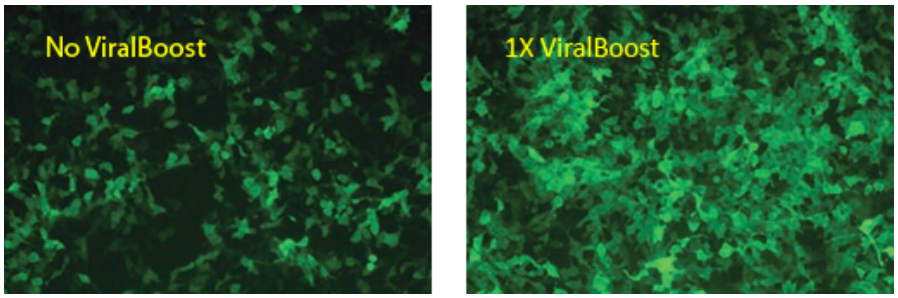
Protocol (VB100)

Results



Flow cytometry data from HEK 293T cells transduced by GFP retroviruses, which were packaged in the absence and presence of the ViralBoost™ Reagent.



HEK 293T cells transduced by GFP lentivirus which were packaged with and without ViralBoost™.

Procedure

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 cell suspensions and to evenly distribute the cells.

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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 tapping the tube. To the other tube, add NanoFect™ (Cat. # NF100). Mix by tapping 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 2-3 times. Mix well by vortexing for 5-10 sec.
4. Incubate for ~15 min at room temperature to allow for NanoFect™/DNA complex 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 1000 x *g* 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. # VC100 & VC200) to every 4 volumes of virus-containing supernatant.
   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 1500 x *g* for 30 min 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 1500 x *g* 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 vary between cell lines. It is advised to test the effect of ViralBoost™ on the target cells beforehand.

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