Introduction

Viral Receptor Booster technology allows you to temporarily increase the density of viral receptor proteins on any cell surface, including cells that are generally resistant to viral infection. Viral receptor proteins are delivered to the cell surface via exosome-like particles. Boosters are available for the amphotropic, CAR and ecotropic receptors:

- The **Ecotropic Receptor Booster (Cat. No. 631471)** allows for the transduction of cells that are resistant to, or inefficiently infected by, ecotropic pseudotyped lentivirus or retrovirus (e.g. human cells). The Ecotropic Receptor Booster delivers the mCAT-1 receptor to target cells.
- The **Amphotropic Receptor Booster (Cat. No. 631469)** increases the surface density of the hPit2 receptor on target cells so that they can be more efficiently transduced with amphotropic retrovirus.
- The **CAR Receptor Booster (Cat. No. 631470)** allows for the transduction of cells that are otherwise resistant to adenoviral infection (e.g. hematopoietic cells) by increasing the surface density of CAR, a target membrane receptor for adenoviruses.

I. General Considerations

A. Storage

Store Receptor Boosters at -70°C upon receipt.

B. Stability

Receptor Boosters are stable for one year from date of receipt under proper storage conditions.

C. Additional Materials Required

The following materials are required but not supplied:

- High titer viral supernatant
- Complete medium
- Polybrene (Hexadimethrine bromide, Sigma Cat. No. H9268)

We recommend the following products for producing virus:

- Ecotropic lentivirus – Lenti-X™ HTX Ecotropic Packaging System (Cat. No. 631251)
- Ecotropic retrovirus – Retro-X™ Universal Packaging System (Cat. No. 631530) or EcoPack™ 2-293 Cell Line (Cat. No. 631507)
- Amphotropic retrovirus – Retro-X Universal Packaging System (Cat. No. 631530) or AmphoPack-293 Cell Line (Cat. No. 631505)
- Adenovirus – Adeno-X™ Adenoviral System 3 (several Cat. Nos.; search www.clontech.com for more information)

D. Cell Plating & Transduction Conditions

Plate your cells one day prior to transduction. They should be 60–80% confluent on the day of viral transduction. Cultures that are less than 50% confluent at the time of transduction may lose viability.

NOTE: Upon first use, we recommend using the highest MOI (multiplicity of infection) possible and a range of Receptor Booster volumes in order to maximize the transduction efficiency for your specific cell type.

II. Procedure

This protocol describes an application using adherent cells cultured in 6-well plates. Suspension cell cultures should be treated at their recommended growth density. Perform the following steps under sterile conditions:

A. Treat Target Cells with Viral Receptor Booster

1. Check that the cells plated on the previous day are about 60–80% confluent.
2. Thaw Receptor Booster at room temperature and keep on ice.*
3. Prepare polybrene (PB) media: Supplement complete media with 4 µg/ml polybrene.
4. Replace the complete media with 2 ml PB media.
5. Add 10–20 µl of Receptor Booster to each well of a six well plate.**
6. Centrifuge the 6-well plate at 1,200 g for 15 min at room temperature.***
7. Incubate the cells for 2 hr at 37°C in an appropriate cell culture incubator.

NOTES:

* Return unused Booster to -70°C freezer. Boosters are stable for at least 10 freeze-thaw cycles.

** Use 5–10 µl of Receptor Booster per well of a 12-well plate.
Use 2.5–5 µl of Receptor Booster per well of a 24-well plate.
We recommend pre-treating your cells with the higher amount of Booster.

*** If a plate centrifuge is unavailable the Boosters will still work, but with less efficiency.

B. Transduce Viral Receptor Booster-Treated Target Cells

1. Replace the Receptor Booster/PB media with 2 ml fresh PB media.
2. Add virus to the target cells at the desired MOI.

NOTE: Receptor Boosters aid in the transduction of difficult-to-transduce target cells by providing an elevated concentration of the membrane receptors to which virus can bind. They cannot compensate for low-titer viral preparations.

3. Centrifuge the virus-treated cells at 1,200 g for 15 min at room temperature.
4. Incubate the cells at 37°C in an appropriate cell culture incubator.
5. After 16 hrs, replace the virus-containing PB media with 2 ml complete media.
6. Continue to cultivate the transduced cells and assay as required.

NOTE: Receptor levels drop to endogenous levels within 48 hr after Receptor Booster treatment.

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Fax: 800.424.1350 (toll-free)	Fax: 800.424.1350 (toll-free)
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