**Description**

IL15/IL15Ra Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles ready to transduce nearly all types of mammalian cells, including primary and non-dividing cells. These particles result in expression of human IL15 (NM_000585.4) and IL15Rα (NM_002189.3) driven by an EF1a promoter, and a puromycin selection marker (Figure 1). IL15 and IL15Ra are fused via a glycine-serine linker.

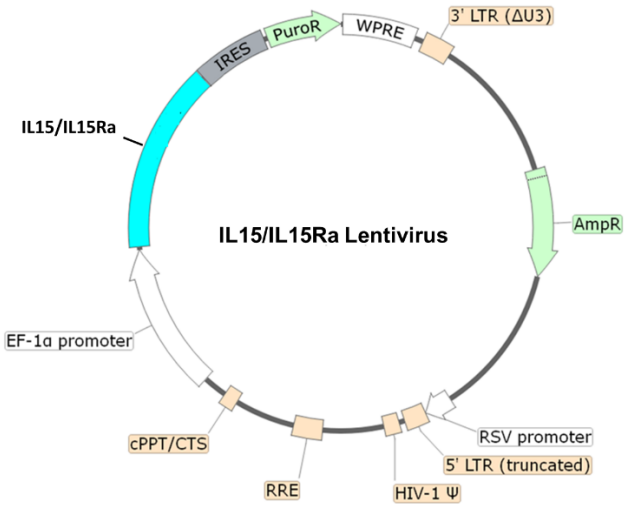


*Figure 1. Schematic of the lenti-vector used to generate IL15/IL15Ra Lentivirus.*

**Background**

IL15 (interleukin 15) and its high affinity receptor (IL15R) are involved in NK cell development and proliferation and persistence of $CD8^+$ T cells, NKT cells, δγT cells and NK cells. The use of membrane bound IL15 as part of the CAR in T cells has been in use for a few years, as a way to improve T cell persistence *in vivo*. IL15 has a short half-life and requires treatment in high dosages, so research into ways to increase its potency has been ongoing. RLI-15 (receptor-linker-IL-15) is considered a super agonist composed of IL15 and an IL15Rα domain. This fusion protein was designed to bypass the need for endogenous IL15Rα to take advantage of IL15. It has been shown to activate proliferation and activity of NK. RLI-15 resulted in a potent effect in cell models. Recently the use of a CAR containing IL15/IL15Rα and targeting CD19 in NK92 cells has also resulted in increased proliferation, cytokine secretion and cytotoxicity towards B-cell cancer cell lines. The inclusion of IL15/IL15Rα fusion proteins to armor CARs represents a step forward in the fight against solid tumors.

**Application(s)**

- Expression of human IL15/IL15Ra in cells of interest.
- Arming CAR-T and CAR-NK cells with IL15/IL15Ra fusion protein.
- Generate cell pools or stable cell lines expressing IL15/IL15Ra following puromycin selection.

**Formulation**

The lentivirus particles were produced in HEK293T cells in medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations and produced at higher titers by special request, for an additional fee.

**Size and Titer**

Two vials (500 µl x 2) of lentivirus at a titer ≥$10^7$ TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

**Storage**

Lentiviruses are shipped with dry ice. For long-term storage, it is recommended to store the lentiviruses at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

**Biosafety**

The lentiviruses are produced with a SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and after integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal and are not present in the lentivirus particle. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS Bioscience recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

**Notes**

To generate an IL15/IL15Ra expressing stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of puromycin (as pre-determined from a killing curve, https://bpsbioscience.com/cell-line-faq), for antibiotic selection of transduced cells, followed by clonal selection.

**Figures and Validation Data**

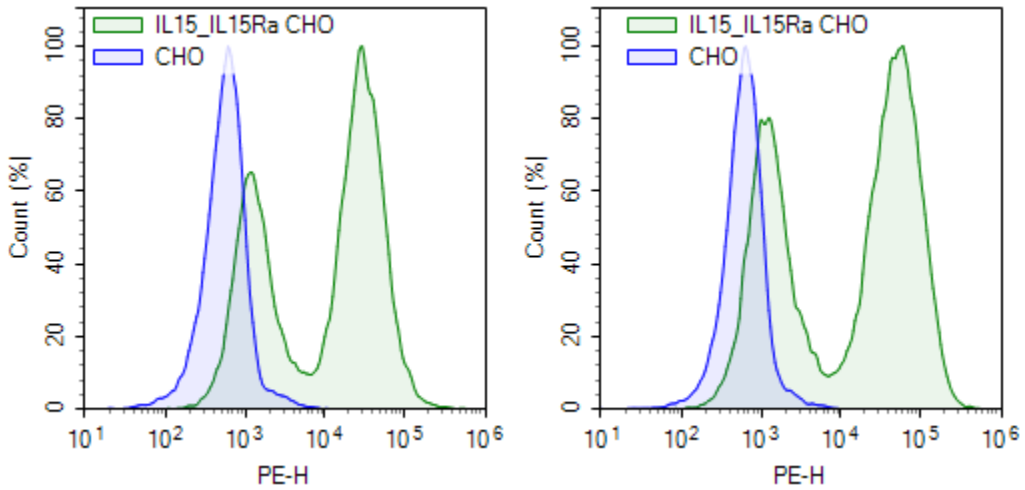


*Figure 2. Expression of IL15 and IL15Ra in CHO cells transduced with IL15/IL15Ra Lentivirus.* Approximately 100,000 CHO cells were transduced with 1 x $10^6$ TU of IL15/IL15Ra Lentivirus in the presence of 5 µg/ml of Lenti-Fuse™ Polybrene Viral Transduction Enhancer (BPS Bioscience #78939). 66 hours post-transduction, cells were stained with IL15 Monoclonal Antibody (34559), PE (ThermoFisher #MA5-23561) (left panel) and CD215 (IL-15Ra) Monoclonal Antibody (eBioJM7A4), PE (ThermoFisher #12-7159-42) (right panel) and analyzed by flow cytometry. The y-axis represents the cell % and the x-axis indicates PE intensity. Non-transduced CHO cells were used as control.

*Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com*

**Sequence**
Human IL15/IL15Ra sequence

MDWTWILFLVAAATRVHSNWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLLELQVISLESGDASIHDTVENLIIL
ANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTSGGGGSGGGGSGGGGSGGGGSGGGSLQITCPPPMSVEHAD
IWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAHWTTPSLKCIRDPALVHQRPAPPSTVTTAGVTPQPESLSPSGKEPA
ASSPSSNNTAATTAAIVPGSQLMPSKSPSTGTTEISSHESSHGTPSQTTAKNWELTASASHQPPGVYPQGHSDTTVAISTSTVLLC
GLSAVSLLACYLKSRQTPPLASVEMEAMEALPVTWGTSSRDEDLENCSHHL

**References**
Hurton L., *et al.*, 2016 *Proc Natl Acad Sci USA* 113 (48): E7788-E7797.
Fujii R., *et al.*, 2018 *Cancer Immunol Immunother.* 67(4):675-689.
Desbois M., *et al.*, 2020 *Journal for ImmunoTherapy of Cancer* 8:e000632.
Silvestre R., *et al.*, 2023 *Front. Immunol.* 14: https://doi.org/10.3389/fimmu.2023.1226518.

**Troubleshooting Guide**
Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For further questions, please email support@bpsbioscience.com.

**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| Firefly Luciferase-eGFP Lentivirus (G418 or Puromycin) | 79980 | 500 µl x 2 |
| Expression Negative Control Lentivirus (EF1A Promoter/ Puromycin) | 82212-P | 500 µl x 2 |
| Lenti-Fuse™ Polybrene Viral Transduction Enhancer | 78939 | 500 µl |
| Human Interleukin-15 Recombinant | 90180 | 2 µg/10 µg |
| IL-15 Responsive Luciferase Reporter Cell Line | 78402 | 2 vials |

*Version 011724*