Lentiviral vectors are increasingly becoming the tool of choice for gene delivery to difficult-to-transfect cells. Currently, production of high titers of lentiviral vectors is a time consuming, multi-step procedure with low reproducibility. To solve these problems, InvivoGen has developed LENTI-Smart™, a novel method to generate high titers of lentiviral vectors, simply, rapidly and efficiently. Depending on your needs, LENTI-Smart™ kits are available for the production of either integrating or non-integrating lentiviral (NIL) vectors. Integrating lentiviruses are best for stable transgene expression. NIL vectors are particularly useful for transient transgene expression in gene therapy protocols and stem cell modifications, where the risk of insertional mutagenesis is a safety concern. This newsletter also features innovative products for the generation of induced pluripotent stem (iPS) cells. These products comprise reprogramming enhancers, plasmids for the production of tagged cell-penetrating reprogramming factors and the multicistronic LENTI-Smart™ OSKM expression vector.

Inside this issue:

**PRODUCTS**
- LENTI-Smart™
  - LENTI-Smart™ (INT)
  - LENTI-Smart™ NIL
  - LENTI-Smart™ OSKM
- Induced Pluripotent Stem (iPS) Cells
  - Reprogramming Factors
  - Reprogramming Enhancers

**SHORT REVIEWS**
- Lentiviral vector production and cell transduction
- Generation of iPS cells
LENTI-Smart™ - Integrating / Non-Integrating Lentiviral Vectors Made Easy

Lentiviral vectors are major tools for gene delivery in mammalian cells, with the ability to mediate potent transduction and stable expression into dividing and non-dividing cells both in vitro and in vivo. LENTI-Smart™ is a lyophilizate of optimized packaging plasmids combined with a DNA transfection reagent which upon rehydration serves as a “carrier” for your favorite lentiviral expression plasmid. LENTI-Smart™ kits are available for the production of either integrating or non-integrating lentiviral (NIL) vectors.

- **LENTI-Smart™ (INT)** allows the generation of integrating lentiviruses for long term expression in dividing and non-dividing cells.
- **LENTI-Smart™ NIL** is the first kit designed for the generation of non-integrating lentiviral vectors, allowing transient transgene expression in dividing cells and long-term expression in non-dividing cells.

**Description**

LENTI-Smart™ is a ready-to-use product that allows for rapid and reliable production of high titers of second generation lentiviral particles in HEK 293T cells. LENTI-Smart™ combines a mix of optimized packaging plasmids precomplexed to a transfection reagent, LyoVec®, selected for its high transfection efficiency and low cell toxicity. This lyophilized complex is provided with a control lentiviral expression plasmid.

**Packaging Plasmids**

The two packaging plasmids forming the LENTI-Smart™ lyophilizate provide the structural and replication proteins in trans that are required for the production of the lentiviral particles.

- **pLV-HELP** expresses the G glycoprotein gene from Vesicular Stomatitis Virus (VSV-G) to allow production of a pseudotyped lentiviral vector with a broad host range.
- **pLV-HELP-NIL D64**, in the LENTI-Smart™ (INT) kit, contains the viral gag, pol, rev and tat genes and the rev-responsive element (RRE).
- **pLV-HELP-NIL D64**, in the LENTI-Smart™ NIL kit, expresses a mutant integrase (D64V) resulting in the generation of lentiviral vectors that are integration defective¹².

**Control Lentiviral Expression Plasmid**

The LENTI-Smart™ kit includes a control lentiviral expression plasmid designed to optimize virus production and cell transduction.

- **pLV-Green** expresses a green fluorescent protein (GFP) gene and contains key viral elements for lentivirus production and safety:

**Contents and Storage**

LENTI-Smart™ (INT) and LENTI-Smart™ NIL are available in 2 sizes, either 5 vials or 10 vials. Each vial allows the transfection of HEK 293T cells with a lentiviral expression plasmid in a 10-cm culture plate or a 75 cm² flask.

A LENTI-Smart™ Starter Kit is also available to allow the generation of both integrating and non-integrating lentiviral vectors. It contains 5 vials of LENTI-Smart™ (INT) and 5 vials of LENTI-Smart™ NIL.

All LENTI-Smart™ kits are provided with a vial of the control lentiviral expression plasmid. The LENTI-Smart™ vials are provided lyophilized, the control plasmid is provided as a liquid. Products are shipped at room temperature and should be stored at -20°C.

User manual and simplified maps are available on our website: [www.invivogen.com/lentismart](http://www.invivogen.com/lentismart)

Sequences of the plasmids are provided upon request.

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**LENTI-Smart™ Procedure**

1. Add lentiviral expression plasmid to LENTI-Smart™ to generate complexes.
2. Add complexes to HEK 293T cells.
3. Harvest viral supernatants 36-48 hours post-transfection.

The unique formulation of LENTI-Smart™ allows to prepare lentiviral expression plasmid / packaging plasmids complexes by simply rehydrating the lyophilizate with the lentiviral expression plasmid solution. There is no need for a transfection reagent as it is included in the LENTI-Smart™ lyophilizate. Transfection of HEK 293T cells is readily performed by adding the complexes to the cells. Lentiviral particles can be collected 2 days after transfection.

Viral titers obtained using LENTI-Smart™ and other transfection reagents with different commercially available lentiviral expression plasmids. Reproducibility is superior with LENTI-Smart™ (reduced standard deviation, n=5).

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**Generation of iPS Cells**

Generation of induced pluripotent stem (iPS) cells by reprogramming adult somatic cells can be achieved by ectopic expression of specific transcription factors. The most widely used set of “reprogramming” factors, Oct4, Sox2, Klf4 and c-Myc, was identified initially by screening 24 pre-selected factors in mouse embryonic fibroblasts (MEFs) by Takahashi and Yamanaka. This cocktail of transcription factors, OSKM, was shown to work for different types of somatic cells and for different species, including rhesus monkey and human cells. Further studies have demonstrated that Oct4 and Sox2 are indispensable whereas Klf4 and c-Myc are dispensable (although the efficiency of iPS cell formation is significantly lower). The generation of iPS cells is usually achieved by genetic transduction of the reprogramming genes using retroviral or lentiviral vectors. However, the use of integrating viral vectors represent an obstacle to the therapeutic translation of iPS cells as this technology can produce insertional mutagenic lesions that are potentially tumorigenic. Two recent publications detail the use of polycistronic lentiviral vectors delivering the OSKM quartet to somatic cells in a single lentiviral construct reducing the number of genomic insertions. Alternative approaches to deliver the reprogramming factors with minimal or total absence of genetic modifications have been developed. These approaches include the use of LoxP sites and Cre-induced excision and piggyBac transposon excision of integrated reprogramming vector sequences, and the use of an on/offBNAI-based episomal vector. Non-integrating lentiviral vectors may also represent a promising approach. One possible strategy to entirely replace gene delivery is protein transduction. Previous studies have demonstrated that various proteins can be delivered into cells by conjugating them with a short peptide that mediates cell penetration, such as polyarginine. Zhou et al. have designed and purified poly-arginine tagged Oct4, Sox2, Klf4 and c-Myc proteins that were found to readily enter cells and translate into the nucleus. After several cycles of protein supplementation, iPS cells were successfully generated from MEFs. Using a similar approach, Kim et al. obtained protein-induced pluripotent stem cells from human newborn fibroblasts after several rounds of treatment with cell extracts of HEK293 cell lines expressing poly-arginine tagged OSKM genes. Substantial research and development is still required before iPS cells are ready for therapeutic applications.


**LENTI-Smart™ OSKM - Generation of iPS Cells**

Induced pluripotent stem (iPS) cells are generated from differentiated somatic cells overexpressing a set of specific transcription factors called reprogramming factors. Typically four reprogramming factors are chosen, Oct4, Sox2, Klf4 and c-Myc (OSKM), which are introduced into the target cells through genetic transduction using retroviral or lentiviral vectors. iPS cells offer exciting possibilities in stem cell research and regenerative medicine. To facilitate the generation of iPS cells, InvivoGen has developed LENTI-Smart™ OSKM for the production of lentiviral vectors expressing the human or murine OSKM reprogramming genes.

**Description**

LENTI-Smart™ OSKM comprises the complexing lyophilize, formed by the packaging plasmids and the transfection reagent, and the lentiviral plasmid expressing the OSKM genes of human or mouse origin.

**Single Reprogramming Expression Cassette**

The four reprogramming genes are expressed from a single multicistronic transcript. This reprogramming cassette contains the coding sequences of Oct4, Sox2, Klf4 and c-Myc separated by three different “self-cleaving” 2A peptides (E2A, P2A, and T2A, respectively).

**Integrating or Non-Integrating Lentiviral Vectors**

Two LENTI-Smart™ OSKM kits are available allowing the generation of integrating or non-integrating lentiviral vectors:

- **LENTI-Smart™ (INT) OSKM** features pLV-HELP which expresses the wild-type integrase for the production of integrating lentiviral vectors
- **LENTI-Smart™ NIL OSKM** features pLV-HELP-NIL which expresses a mutant integrase for the production of non-integrating lentiviral (NIL) vectors, designed for transient transgene expression.


**Induction of pluripotent stem cells: Somatic cells are obtained from adult organism. The reprogramming factors are introduced into the cultured somatic cells. The cells are grown under ES cells conditions. After 2-3 weeks, iPS cells emerge. These induced pluripotent stem cells may be differentiated into various cell types for regenerative medicine applications.**

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Poly-Arginine-HA Tagged Reprogramming Factors

Description

Poly-arginine-HA tagged reprogramming factors allow the production of recombinant cell-penetrating reprogramming factors. They correspond to the four transcription factors, Oct4, Sox2, Klf4, and c-Myc (OSKM), fused at their C terminus to a poly-arginine (i.e. 11R) peptide in tandem with 3 motifs of the hemaglutinine (HA) tag. The poly-arginine peptide enables the recombinant proteins to readily enter the cells and have been shown to allow their translocation into the nucleus 1, 2. The HA tag is useful for their detection by Western blot or their purification by affinity chromatography.

Poly-arginine-HA tagged reprogramming factors are cloned in the pUNO1 plasmid within a mammalian expression cassette comprising the EF-1 *a* /HTLV composite promoter and the SV40 poly adenylation sequence. pUNO1 plasmids are selectable in *E. coli* and mammalian cells with blasticidin.


Applications

Poly-arginine-HA tagged reprogramming factors are designed for the generation of protein-induced pluripotent cells. Following their transfection into mammalian cell lines, such as HEK293, the cell extracts can be used crude to treat the target cells or processed to purify the poly-arginine-HA-tagged proteins on an anti-HA affinity column.

For native reprogramming factors in the pUNO1 plasmid, see www.invivogen.com/ipsc

Contents and Storage

Each pUNO1 plasmid is provided as a lyophilized transformed *E. coli* strain on a paper disk. Transformed strains are shipped at room temperature and should be stored at -20°C.

Reprogramming Enhancers

Direct reprogramming of somatic cells is currently a slow and inefficient process, in particular when the c-Myc oncogene is omitted in an effort to reduce tumorigenicity. Several chemicals have recently been reported to either enhance reprogramming efficiencies or substitute for specific reprogramming factors. Among the reported chemicals, some are known to affect chromatin modifications while others influence signal transduction pathways.

Small molecules that modulate chromatin modifications include the DNA methyltransferase inhibitor (5-azacytidine), the histone deacetylase inhibitor (valproic acid) and a G9a histone methyltransferase (Bix-01294) 1-3. The MEK inhibitor PD035901 and the TGF-β receptor inhibitor SB431542 potentiate reprogramming by targeting signaling pathways 4.


Contents and Storage

Each product is provided as a solid and shipped at room temperature. Store at room temperature, 4°C or -20°C according to the product label.

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InvivoGen

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