3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Integration Deficient Lentivirus (IDLV) Packaging Mix

Catalog Number I3411
Storage Temperature –20°C

TECHNICAL BULLETIN

Product Description

The Integration Deficient Lentivirus (IDLV) Packaging Mix is an optimized formulation of plasmids expressing the key HIV packaging genes and a heterologous viral envelope gene. A novel feature of the IDLV Packaging Mix is a packaging vector including a mutation (D64V) in the gene encoding the viral integrase protein. This mutation reduces viral genomic integration rates by several orders of magnitude (Leavitt, et al., 1996; Wanisch, et al., 2009) enabling transient episomal expression of genes via lentiviral delivery. Key applications for transient gene expression have been the delivery of zinc finger nucleases (ZFNs) for genome editing (Lombardo, et al., 2007; Provasi, et al., 2012) and Cre protein for recombinase mediated cassette exchange (Torres, et al., 2011).

IDLV particles are generated from three main components:

1. The packaging vector, which contains the minimal set of lentiviral genes required to generate the virion structural proteins and packaging functions
2. The vesicular stomatitis virus G-protein (pCMV-VSV-G) envelope vector, which provides the heterologous envelope for pseudo-typing
3. The transfer vectors, such as Sigma Aldrich ZFN IDLV-FP constructs in products CKOZV1, CSTZV1 or ZFN IDLV-FP non-targeted control ZVCTRLD, which contain the ZFN sequence of interest as well as the *cis* acting sequences necessary for RNA production and packaging. Non-ZFN vectors harboring other transgenes may also be used with this packaging mix to produce IDLV particles for a variety of applications

The IDLV Packaging Mix contains the first two components; it is designed to be co-transfected along with a compatible IDLV transfer vector in order to create high-titer IDLV particles used for downstream transduction applications.

In this packaging system, the pseudo-typing with VSV-G broadens the viral tropism associated with the virus (Burns et al., 1993). Therefore, these IDLV particles can efficiently deliver the transfer sequence of interest to a wide variety of cell types, including primary and non-dividing cells.

The particles are produced using a third-generation packaging system, which has many features that lead to enhanced biosafety (Dull, et al., 1998). Some of these features are described in Table 1.

Table 1.
Biosafety Features of the Third-Generation Integration Deficient Lentivirus Packaging System

Feature	Result
Multi-plasmid approach	No single plasmid contains all the genes necessary to produce packaged IDLV Resultant particles are integration and replication-incompetent
Deletion in U3 portion of 3' LTR which eliminates the promoter-enhancer region	Avoids promoter interference issues and further negates the possibility of viral replication
Elimination of the majority of lentiviral genes (Δ vpr, vif, vpu and nef) [5]	Removes virulence genes which are not necessary for packaging systems

The IDLV Packaging Mix and the IDLV ZFN transfer vectors can be easily co-transfected into a mammalian production cell line. We recommend HEK293T cells, ATCC® Number CRL-11268, as they are readily transfected and have been demonstrated to consistently produce a high viral titer (Pear et al., 1993). Transduction efficiency in IDLV format has been shown to be reduced (7-17 fold) relative to integrating-lentiviral formats (Wanisch et al., 2009). Higher multiplicity of infection (MOI) may be necessary for sufficient gene expression. For specific IDLV-ZFN protocol recommendations, please see the CompoZr ZFN Technical Bulletin.

Lentivirus produced with the IDLV Packaging Mix has not been shown to produce replication competent viral

particles because of designed safety features. Users should consult and observe their own institutional guidelines for working with such viral systems.

Reagents
Integration Deficient Lentivirus (IDLV) Packaging Mix
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Reagents and equipment required, but not provided

- Integration-deficient lentiviral-based transfer vector(s).
- TransIT®-LT1 Transfection Reagent
  Mirus Bio LLC - Cat. No. MIR 2304
- HEK293T producer cells
  ATCC - Cat. No. CRL-11268
- Serum-free DME medium
  Sigma - Cat. No. D6171
- Fetal Bovine Serum (10%)
  Sigma - Cat. No. F4135
- L-glutamine (4mM)
  Sigma - Cat. No. G5713
- Sodium Pyruvate (1mM)
  Sigma - Cat. No. S8636
- MEM Non-essential amino acid solution (1%)
  Sigma - Cat. No. M7145
- Penicillin-Streptomycin (optional) (1%)
  Sigma - Cat. No. P4333
- HIV-1 p24 Antigen ELISA Kit,
  ZeptoMetrix - Cat. No. ZMC0801200

Follow distributor's instructions for culturing and maintaining the producer cell line. Cells should be in log phase at the time of transduction and should not be used immediately after thawing or at very high passage numbers.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Though the lentiviral transduction particles produced are replication incompetent, it is highly recommended that they be treated as **Risk Group Level 2 (RGL-2)** organisms.[7] Follow all published RGL-2 guidelines for handling and waste decontamination. Also, use extra caution when packaging IDLV particles that express targeting genes involved in cell cycle control, e.g., tumor suppressor genes.

Storage/Stability
If stored properly, shelf life is 2 years from the date of shipment. The IDLV Packaging Mix should be stored at –20 °C. Avoid multiple freeze thaw cycles.

Procedure Overview

Day 1	Day 2	Day 3	Day 4	Day 5
Plate HEK293T cells	Transfect cells with packaging mix and transfer vector	Re-feed cells with fresh media	IDLV particle harvest. Start p24 assay	End p24 assay

PROCEDURE
Note: The following procedure was validated in a T225 flask format.

Day 1
Plate HEK293T cells (**13.5 x 10^6 cells/flask**) in complete DME media 24 hours prior to transfection. Cells should reach 70%-80% confluency the day of transfection.

Day 2
1. Thaw the vial of IDLV Packaging Mix at room temperature. Bring Serum-Free DME and Transfer Vector to room temperature. Keep transfection reagent at 4°C until ready to use.
2. Label sterile polypropylene tube(s) for each transfection. Add the reagents in the given order in a sterile environment.
   - Serum-free DME **352.9 µL**
   - Packaging Mix **107.0 µL**
   - Transfer Vector **10.71 µg**
   - Transfection Reagent **64.20 µL**
3. Mix components gently by pipetting up and down.
4. Let sit for 15-45 minutes at room temperature.
5. Add entire transfection cocktail volume to the corresponding flask(s) containing HEK293T cells.
6. Incubate at 37 °C for 12-24 hours.

Day 3
Change media
7. Pre-warm a sufficient amount of complete media to 37 °C to be used for feeding the cells.
8. At 12-20 hours post-transfection, remove media from the transfected cells (avoid disturbing cells) and replace with 35 mL of the pre-warmed complete media.
9. Incubate cells in incubator (37 °C and 5% CO_2) for an additional 24 hours.

Day 4

Harvest of IDLV Particles

10. Collect IDLV particles by carefully removing all the media and placing it in an appropriately sized sterile container(s).
11. Discard cells immediately and clarify the harvest using a low-protein binding 0.45 μm filter (Millipore SLHV033RS).
12. Perform a high-speed spin to pellet the IDLV harvest.
    a. Balance the tubes by adding cold media if needed.
    b. Spin for 1-2 hours at 50,000xg at 4 °C.
13. Aspirate media very carefully.
    Note: The IDLV pellet may be very small and hard to visualize. On the outside of the tube, mark the spot of the pellet and aspirate on the opposite side.
14. Resuspend pellet in desired volume of complete media (~300 μL for most IDLV applications). Aliquot into desired volumes for use.
    Note: IDLV particles can be stored at 2-8 °C for 24-48 hours. For long-term storage, freeze at –70 °C. Multiple freeze thaw cycles of viral particles may reduce the infectious viral titer by 20-50% per cycle.
    Titer viral particles by performing the HIV p24 Antigen ELISA assay immediately after pooling (see manufacturer's protocol).

References

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6. Pear, W.S., *et al*., Production of high-titer helper-free retroviruses by transient transfection. *Proc. Natl. Acad. Sci. USA*, **90**, 8392-8396 (1993).
7. Provasi et al., Editing T cell specificity towards leukemia by zinc finger nucleases and lentiviral gene transfer. *Nat. Medicine*. 18(5)807-15 (2012).
8. Torres et al., Non-Integrative Lentivirus Drives High-Frequency Cre-Mediated Cassette Exchange in Human Cells. PloS One. 6(5):e19794 (2011).
9. Wanisch et al., Integration-deficient Lentiviral Vectors: A Slow Coming of Age. *Mol. Ther.* 17(8):1316-32 (2009).

Troubleshooting Guide

Problem	Cause	Solution
Low viral titer or no viral titer determined in HIV p24 Antigen ELISA assay	IDLV Packaging Mix was not added to the transfection mix.	Repeat experiment.
	IDLV Packaging Mix was not stored properly.	Check if IDLV Packaging Mix was stored at –20 °C upon receiving.
	IDLV Packaging Mix went through multiple freeze-thaw cycles.	After receiving, generate aliquots of the IDLV Packaging Mix and store at –20 °C until ready for use.
	The target gene is essential for cell growth and viability.	Be sure that target gene is not essential for cell growth and viability.
	Vector construct is too large.	The recommended size between 5' and 3' LTRs is <10Kb. Larger regions can result in lower or no IDLV titers.
	Low yield of target vector plasmid DNA.	The viral-based vectors are known to be difficult to purify. We recommend streaking the bacterial stock on LB/carbenicillin plate to isolate a single colony and DNA purification with GenElute™ HP Midiprep Kit, Catalog Number NA0200, or GenElute HP Maxiprep Kit, Catalog Number NA0300, or GenElute HP Plasmid Miniprep Kit, Catalog Number NA0160 or NA9604.
	Used poor quality plasmid DNA.	Use DNA purification with Sigma's GenElute plasmid purification kits mentioned above.
	Too much transfer DNA was added to the transfection mix.	Verify DNA concentration using OD or PicoGreen® quantification method. Make sure correct volume of DNA was added during transfection.
	Seeding density of cells was not optimal.	Follow cell density recommendations (refer to surface area). Be sure HEK293T cells are between passages 2 and 20, in a healthy culture and in log phase at the time of plating.
	Media and reagents were stored improperly.	Store media and reagents at temperatures designated by the manufacturers.

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