

Product Analysis Certificate

Ready-to-Use Packaging Plasmid Mix
Cat.# CPCP-K2A



Ready-to-Use Packaging Plasmid Mix

Product: Ready-to-Use Packaging Plasmid Mix

Catalog #: CPCP-K2A

Lot #: 15103001

Description:

The Ready-to-Use Packaging Plasmid Mix is a mixture of the human immunodeficiency virus (HIV) lentiviral packaging plasmid psPAX2 (Addgene, Cat.# 12260) and the pMD2.G plasmid (Addgene, Cat.# 12259) containing VSV-G. These plasmids provide all the necessary structural, regulatory, and replication proteins required to produce pseudotyped packaged lentiviral expression constructs when co-transfected with an HIV-based lentiviral expression construct (*e.g.* Collecta pRSI shRNA expression construct) into a producer 293T cell line (*e.g.* ATCC, Cat.# CRL-11268™). Pseudotyped lentiviral particles with VSV-G envelope protein can infect a variety of mammalian or non-mammalian, dividing or non-dividing cells. The kit contains 250 µg of lentiviral packaging plasmid mix that will be sufficient for 25 transfections in 10-cm culture plates.

The Ready-to-Use Packaging Plasmid Mix is compatible with most commercially-available second- and third-generation lentiviral vectors.

Biosafety Level: BSL-2

Storage: -20°C

Shelf Life: 2 years from date of receipt with proper storage

Shipping Conditions: Blue Ice or Dry Ice

Contents:

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1	CPCP-K2A	Ready-to-Use Packaging Plasmid Mix 250 µg, 0.5 µg/µl (500 µl × 1 tube)

Quality Control:

10 µg of Ready-to-Use Packaging Plasmid mix and 2 µg of pRSI16-U6-(sh)-UbiC-TagRFP-2A-Puro were co-transfected into ATCC 293T/17 (Addgene Cat.# CRL-11268™) producer cells with Lipofectamine® Reagent and Plus™ Reagent (Invitrogen) in a 10-cm culture plate as described in the included **Packaging of Individual Lentiviral Constructs** protocol. After transformation, the pRSI16 viral titer was $>1 \times 10^6$ TU/ml as determined by FACS analysis of 293T/17 cells infected by the pseudoviral supernatant.

APPROVED BY: 

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PROTOCOLS

Please visit Collecta's website for the latest protocols:

<http://www.cellecta.com/resources/protocols/>

Technical Support

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Safety Guidelines

The HIV-based lentivector system is designed to maximize its biosafety features, which include:

- A deletion in the enhancer of the U3 region of 3'ΔLTR ensures self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of the target cells.
- The RSV promoter upstream of 5'LTR in the lentivector allows efficient Tat-independent production of viral RNA, reducing the number of genes from HIV-1 that are used in this system.
- Number of lentiviral genes necessary for packaging, replication and transduction is reduced to three (gag, pol, rev). The corresponding proteins are expressed from different plasmids lacking packaging signals and share no significant homology to any of the expression lentivectors, pVSV-G expression vector, or any other vector to prevent generation of recombinant replication-competent virus.
- None of the HIV-1 genes (gag, pol, rev) will be present in the packaged pseudoviral genome, as they are expressed from packaging plasmids lacking packaging signal—therefore, the lentiviral particles generated are replication-incompetent.
- Pseudoviral particles will carry only a copy of your expression construct.

Despite the above safety features, use of HIV-based vectors falls within NIH Biosafety Level 2 criteria due to the potential biohazard risk of possible recombination with endogenous viral sequences to form self-replicating virus or the possibility of insertional mutagenesis. For a description of laboratory biosafety level criteria, consult the Centers for Disease Control Office of Health and Safety Web site at:

http://www.cdc.gov/biosafety/publications/bmbl5/bmbl5_sect_iv.pdf

It is also important to check with the health and safety guidelines at your institution regarding the use of lentiviruses and follow standard microbiological practices, which include:

- Wear gloves and lab coat at all times when conducting the procedure.
- Always work with pseudoviral particles in a Class II laminar flow hood.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory area are to be placed in a durable, leakproof, properly marked (biohazard, infectious waste) container and sealed for transportation from the laboratory.

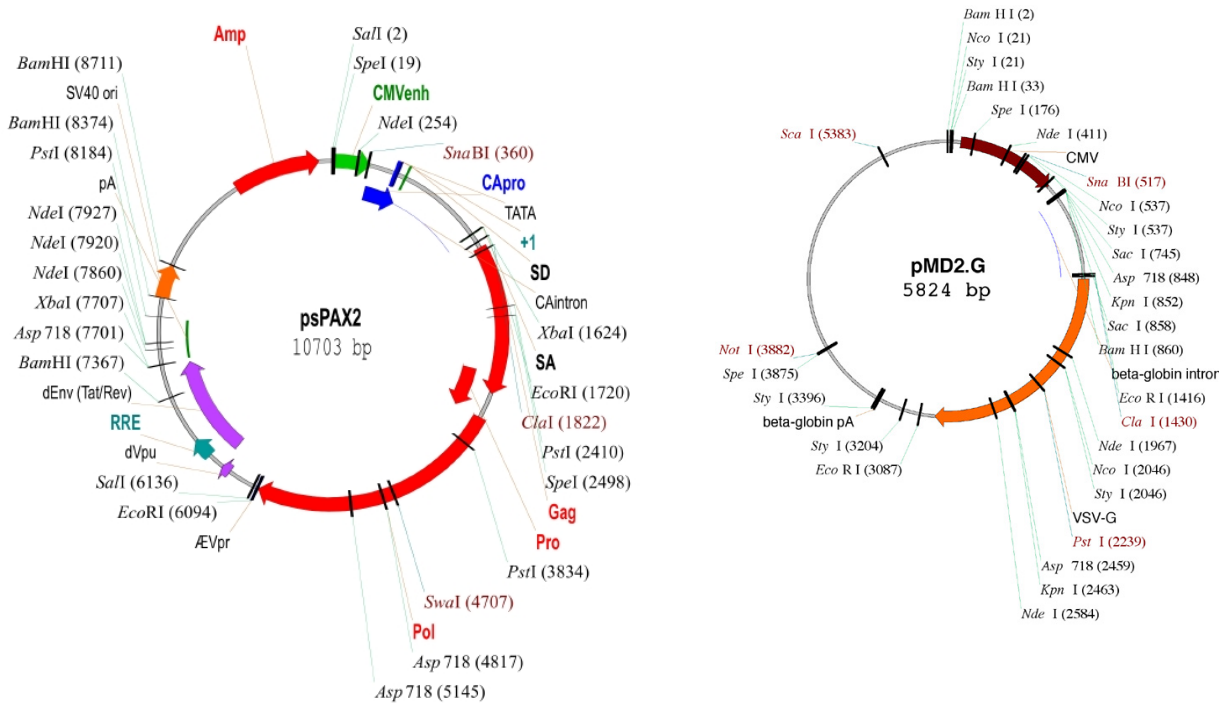
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Appendix

1. Packaging Plasmid Maps



For detailed maps, sequences, feature maps, and restriction maps for psPAX2 and pMD2.G, please see the Addgene website:

psPAX2: <http://www.addgene.org/12260/>

pMD2.G: <http://www.addgene.org/12259/>

For maps, sequences, and cassette designs for standard lentiviral shRNA expression vectors, please visit <http://www.cellecta.com/resources/vectors/>

For all other vectors, please contact Cellecta at tech@cellecta.com.

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