Datasheet for ABIN4368370

Lenti-vpak Packaging Kit

15 Publications

Overview

Quantity:	10 reactions
Application:	Transfection (T)

Product Details

Purpose:	Lenti-vpak packaging system is designed to optimize the packing of most third generation lentivectors into virus particles which can express your lentiviral construct in a multitude of mammalian cells.
Specificity:	- Generate high titers of viral particles - Transfection reagent included
Characteristics:	Lentiviral vectors as gene delivery tool are modified from HIV-1, with most of the viral genes removed. The lentiviral vector only contain the LTRs and the packaging signal, Psi. Lentiviral packaging genes are provided on separate plasmids, so the pseudo lentiviral particles are replication deficient.
Components:	Packaging Plasmids - lyophilized 60 ug (5 x 60 ug) TurboFectin Transfection Reagent - liquid 400 uL (5 x 400 uL)
Material not included:	HEK 293T Cells, Opti-MEM, 0.45 micron filter

Application Details

Application Notes:	Reagent Requirement by Vessel Type: 10 cm dish (2.5x10^6 cells) - 5 µg shRNA Plasmid, 6 µg Packaging Plasmid, 33 µL Transfection Reagent, 1.5 mL Opti-MEM per dish - 10 reactions per Kit 6-well plate (5x10^5 cells) - 1 µg shRNA Plasmid, 1.2 µg Packaging Plasmid, 6.6 µL Transfection Reagent, 250 µL Opti-MEM per vial - 50 reactions per Kit

Application Details

	12-well plate (2.5x10^5 cells) - 0.5 µg shRNA Plasmid, 0.6 µg Packaging Plasmid, 3.3 µL Transfection Reagent, 100 µL Opti-MEM per vial - 100 reactions per Kit
Comment:	For Lenti shRNA application, we recommend a 6-well plate format.
Protocol:	Lenti-vpak Packaging Kit Protocol Protocol below is a for a 10cm dish, see table for different vessels and reagent requirements. Day 1. Plate 2.5 x 10^6 of 293T cells on a 10cm dish in 10 mL complete growth media (antibiotic-free preferred) and incubate at 37 °C overnight. Day 2. Transfection, 1) In a labeled ependorf tube, dilute the following DNA in 1.5mL Opti-MEM, and pipet gently to mix completely. a. 5 µg of pLenti-shRNA construct or 5 µg of pLenti-ORF expression construct b. 6 µg of packaging plasmids 2) Add 33µL of TurboFectin transfection reagent to the diluted DNA (not the reversed order), pipet gently to mix completely. 3) Incubate for 15 min at room temperature. 4) Add the transfection mixture prepared above dropwise to the cells. Gently rock the plate back-and-forth and from side-to-side to distribute the complex evenly. Incubate at 37°. Note: With TurboFectin, no medium change is necessary, directly add the transfection mixture to cells in complete growth media. Day 3. Change the culture medium after 12-18 hours of incubation. Day 4. Harvest the first batch of viral supernatant from the culture and store it at 4 °C. Add 10 mL fresh culture media to the cell culture. Day 5. Harvest the second batch of viral supernatant then combine it with the first batch. Filter through a 0.45µm filter to remove cellular debris. The viral titer at this step is usually 10^6-10^7 TU/mL. The viral supernatant is now ready for the majority of transduction applications. If necessary, further concentration can be applied. Note: Large ORF inserts might decrease the viral titer.
Reagent Preparation:	Packaging Plasmids - Add 120 µL of distilled sterilized water (0.5 µg/µL). Please store at -20°C.
Restrictions:	For Research Use only

Handling

Precaution of Use:	Although the lentiviral transduction particles produced are replication incompetent, it is highly recommended that they be treated as Risk Group Level 2 (RGL-2) organisms. Follow all published RGL- 2 guidelines for handling and waste decontamination.
Storage:	-20 °C
Storage Comment:	store packaging plasmid and transfection reagent at -20°C

Publications

Product cited in:	Johnson, Drugan, Miller, Evans: "38" in: , Vol. 1363, Issue Nucleic acids research, pp. 28-39, (1991)