Data sheet

pASSEMBLE™ Lentiviral Packaging System

Cat. No: ME0044
Cat. No: ME0044-Plus
50 transfection reactions in wells of 6-well plates.

Description

To produce virions, once cloned your gene of interest into a viral vector (known as transfer vector), you need to use of packaging viral vector(s). Transfer vectors have been modified to reduce the hazardous level and highest generation of transfer vectors are lacking of *gag*, *pol*, *env* viral genes. Packaging vectors provide all the viral proteins required for transcription and packaging of your expression cassette into recombinant viral particles.

ASSEMBLE Lentiviral Packaging System includes an optimized mix of three vectors with sequences of *gag*, *pol* and *rev* genes from human immunodeficiency virus (HIV-1) and the envelope gene from vesicular stomatitis virus (VSV-G). VSV-G envelope confers a wide range of tropism because of this glycoprotein binds to phospholipid receptor universally expressed in mammalian cells. This packaging system requires lentiviral transfer vectors of 3rd generation or higher.

Biosafety Features

- ✓ Biosafety Level 2 (BL-2).
- ✓ Mix of three packaging vectors. One of them contains *gag*/ *pol* genes; other, *env* gene and the third, *rev* gene.
- ✓ Rev regulation sequence is located on *gag*/ *pol* expression vector adding an additional biosafety element: in the absence of rev, gag/ pol are not produced.
- ✓ Viruses from SIN vectors result in the transcriptional inactivation of the provirus in the infected cell.
- ✓ Disposable Packaging vectors are 3´LTR SIN and packaging signal deleted (ΔΨ–GAG).

Kit Components

Components	ME0044	ME0044-Plus
pASSEMBLE™ Lentiviral Packaging Mix (0.5 µg/µL)	150 µL	150 µL
CANFAST Transfection Reagent	-	0.5 mL
eGFP Lentiviral Transfer Control Vector (0.5 µg/µL)	-	30 µL

The Kit is enough for 50 transfection reactions in wells of 6-well plates.

Storage

If stored properly, shelf life is 1 year from the date of shipment.
The pASSEMBLE™ Lentiviral Packaging Mix and eGFP Lentiviral Transfer Control Vector should be stored at –20 °C. Avoid multiple freeze thaw cycles.
Important: Store CANFAST Transfection Reagent at 4°C. **DO NOT FREEZE.**

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Protocol

Culturing Packaging Cell Lines

We recommended use HEK-293 cell line to produce viral supernatant.

Virus Production (Protocol for 6-well plate)

This protocol is for 6-well plates, this protocol can be scaled up or down if desired.

1. **For transfection of lentiviral vector, seed your packaging cell line** at 60-80% confluence the day before transfection. The number of cells recommended for 6-well plates are 2.5-8.0x 10^5 cells/well.
2. On the day of transfection, **prepare Transfection Mix as follows:**

   For each well of 6-well plates prepare:

   A. **DNA solution**: Dilute 0.5 µg DNA plasmids and 1.5 µg Lentivirus Packaging Mix in 100 µl Medium without serum and mix gently.

   B. **CANFAST solution**: Dilute 6 µL of CANFAST Transfection Reagent in 100 µl Medium without serum and mix gently.

   C. Prepare the **Transfection Mix** adding **CANFAST solution** drop to drop into **DNA solution** which is gently stirring at vortex.

Tissue Culture Vessel	DNA Solution		CANFAST solution		Transfection Mix
	DNA	Medium without serum	CANFAST Reagent	Medium without serum	
1 well of a 6-well plate	2 µg	100 µL	6 µL	100 µL	200 µL

3. Incubate **Transfection Mix** 15-20 minutes at room temperature. Add **Transfection Mix** into each well by leaking and shake **gentle** the plate.
4. Incubate for 48 – 72 hours to obtain high viral titer in the supernatant (*usually titers of 10^5 – 10^6 cfu/mL*).
5. Collect the viral supernatant and centrifuge by low speed (about 500 x g) for 5 min at 4ºC or pass it through a 0.45 µm cellulose acetate or PES filter for removing cell debris. (Do not use a nitrocellulose filter because the virus is destroyed). Aspirate the supernatant and storage it at -80ºC or infect target cells.

Viral Infection of Adherent Cells (Protocol for 6-well plate)

1. **Plate the cells,** the day before infection, at a cell density of 60-70% confluence. The number of cells recommended for 6 well plates are 2.5-5.0x 10^5 cells / well.
2. On the day of infection, removed from the culture medium of the well and **add viral supernatant.** Use 2ml viral supernatant/well or if you know the viral titer, use the volume of viral supernatant which you need and complete with complete medium up to 2ml/well. If you need improve yours results, you can do multiple rounds of infection. Allow cells to rest for 12-24 hr between each infection.
3. **Add polybrene** to a final concentration of 8 µg/mL. Polybrene range is 2 – 12 µg/mL. Could be necessary optimized this parameter.
4. **Replace medium** with fresh medium after 12-24h of infection. You can replace medium after 4 – 6hr after infection if your target cells require it.
5. After 48- 72hr infection you can analyse the results of infection process.

Viral Infection of Suspension Cells (Protocol for 6-well plate)

1. **Collect the cells** by centrifugation (250g for 5 min) and **resuspend** 1x10^6 cells in 1 mL of the filtered viral supernatant or if you know the viral titer, use the volume of viral supernatant which you need and complete with complete medium up to 1ml.
2. **Add polybrene** to a final concentration of 8 µg/ mL and dispense the mix into a well of the 6-well plate. Polybrene range is 2 – 12 µg/mL. Could be necessary optimized this parameter.
3. **Centrifuge the plate** at 1000g, 1 hr 32ºC (*32ºC increases viral half-life*).
4. **Incubate** the plate for 12-24 hr.
5. **Collecting cells and replace medium** with 2mL of fresh medium by centrifugation (250g for 5min) and **incubate** the cells at 37ºC, 5% CO_2.
6. After 48- 72hr infection you can analyse the results of infection process.

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