

Transfection kit for lentiviral production

The following protocol sets the basis to produce lentiviral particles in 24-well plates as well as 10 cm cell culture dishes using HYVIR at ratio 3:1, 3 μ L of HYVIR per μ g DNA. HYVIR Boost is added at 1X final.

1. Prepare the cells: 24 H before transfection seed the cells in 24-well plate or 100 mm dish.



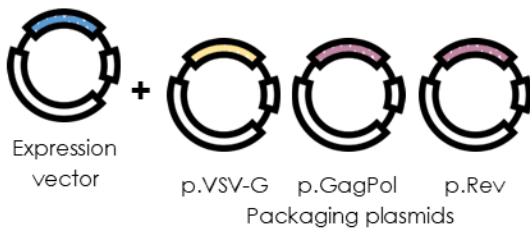
24-well: 5 x 1.10⁴ cells
100 mm: 2 x 1.10⁶ cells



Cells should reach 80-90% confluence on the day of transfection

1 h before transfection, replace medium with fresh pre-warm medium

2. Prepare the lentiviral vectors system

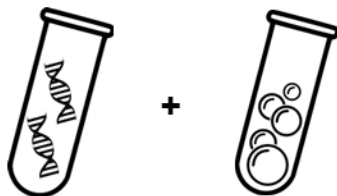


Plasmids mix
2 to 4 plasmids

DNA suspension: mix plasmids necessary for virus production

24-well: 0.3 μ g
100 mm: 3.0 μ g

3. Preparation of the complexes



Plasmids mix
0.3 to 3 μ g

HYVIR
0.9 to 9 μ L



complexes



25 min at RT



Incubate 48 H to 72 H



HYVIR Boost
1X final

4. Collect Supernatant, clarify and titer



Collect supernatant containing Lentiviral particles 48 to 72 H after transfection



Filter through 0.45 μ m



Centrifuge 3500 rpm x 10 min

Clarify lentiviral supernatant

5. Optional

- To increase viral titer, collect supernatant after 48 H, add fresh medium and collect again after 72 H.
- Capture and concentrate viral particles using **Mag4C-LV** or **Viro-PEG** (OZ Biosciences — Not provided with this kit)

Before you begin

HYVIR™ is a novel lipid-based transfection kit specifically designed to enhance viral particle production in HEK-293T cells. Issued from the catalogue of proprietary last generation of lipids from OZ Biosciences, this reliable and reproducible transfection reagent is easily scalable for production in large volumes and can be raised to superior grade.

Cat. Nb	Volume (mL)	HYVIR Boost 100X volume (mL)	Nb of transfections / 24-well plate ¹	Nb of transfections / 100 mm plate ¹
HYV01500	1.5 mL	5 mL	1600	160
HYV03000	2 x 1.5 mL	2 x 5 mL	3200	330
HYV15000	15 mL	50 mL	/	/
HYV30000	2 x 15 mL	2 x 50 mL	/	/

¹Values are given for transfection using the recommended transfection conditions (0.3 µg DNA/well and 3 µg DNA/100 mm dishes).

Table 1: HYVIR kits description and number of transfections.

Protocol | General considerations

- Allow reagents to reach room temperature
- Use medium w/o any supplement for preparation of complexes
- Collect supernatant containing lentiviral particles 48H and/or 72H after transfection. Ideally use the Viro-PEG lentivirus concentrator kit (ref #LVG500) or Mag4C-LV (ref #LKC11000) to increase viral titer.

1. Cells preparation:

One day before transfection prepare the cells according to table 2

Cell culture format	Surface area per well ¹	Cell Nb.	Culture medium (mL)	Vol. for complexes (µL)
24-well	2 cm ²	5 x 1.10 ⁴	0.4	100
100-mm	55 cm ²	2 x 1.10 ⁶	10	200

¹Surface area may vary depending on the manufacturer.

Table 2: Cell number suggested per well.

2. Standard protocol:

Perform a medium change 1H before transfection

The following protocol sets the basis to produce lentiviral particles in 24-well plates as well as 10 cm cell culture dishes using HYVIR, refer to table 3 below for recommended DNA amount and reagent volumes.

Cell culture format	DNA quantity	HYVIR volume	Total volume	Boost volume (1X final)
24-well	0.3 µg (50 µL)	0.9 µL (50 µL)	500 µL	5 µL
100-mm	3 µg (100 µL)	9 µL (100 µL)	10 mL	100 µL

Table 3: Recommended HYVIR volumes and DNA quantities.

NOTE: do not perform a medium change after 24 H as this would reduce the viral titer.

a. Plate the cells **24 H** before the experiment to reach **80-90%** confluence on the day of transfection.

b. Replace medium **1 H** before transfection.

c. Prepare a mix of plasmids by composing packaging and expression plasmids depending on their recommended stoichiometry.

d. Add **0.3 to 3 µg** of total plasmid mix to **50 to 100 µL** medium without any supplement.

e. Add **0.9 to 9 µL HYVIR** to **50 to 100 µL** medium without any supplement.

f. Form complexes by combining DNA and HYVIR solutions.

g. Incubate **25 min** at RT.

h. After incubation, add complexes directly to cells in a dropwise manner, add boost (**1X final**).

i. Incubate **48 to 72 H** and proceed to harvest and concentration or storage of Lentivirus.

Our technical team is at your disposal for any questions or optimization procedures:

✉ - tech@ozbiosciences.com

Use, handling and storage

For Research Use Only. Not for use in humans. Not for use in diagnostic or therapeutic purposes.

Shipping conditions: Room Temperature

Storage conditions HYVIR: +4°C | **HYVIR Boost:** -20°C

Shelf life: 1 year from the date of purchase

We recommend minimizing freeze-thaw cycles to preserve HYVIR integrity.

Kit contents

HYV01500: 1.5 mL of HYVIR + 5 mL of HYVIR Boost

HYV03000: 2 x 1.5 mL of HYVIR + 2 x 5 mL HYVIR Boost

HYV15000: 15 mL of HYVIR + 50 mL of HYVIR Boost

HYV30000: 2 x 15 mL of HYVIR + 2 x 50 mL of HYVIR Boost

Certificate of analysis on demand.

Related Products

Ref	Description
#HY01500	HYPE-5, bioproduction in CHO-S & HEK-293-S
#HY29315	HYPE-293, bioproduction in 293-S
#HYV01500	HY-VIR transfection reagent for viral production

Purchaser Notification | Conditions of Sale

This product is sold in accordance with our general conditions of sale that you can find on our website: <https://ozbiosciences.com/content/3-terms-and-conditions>.