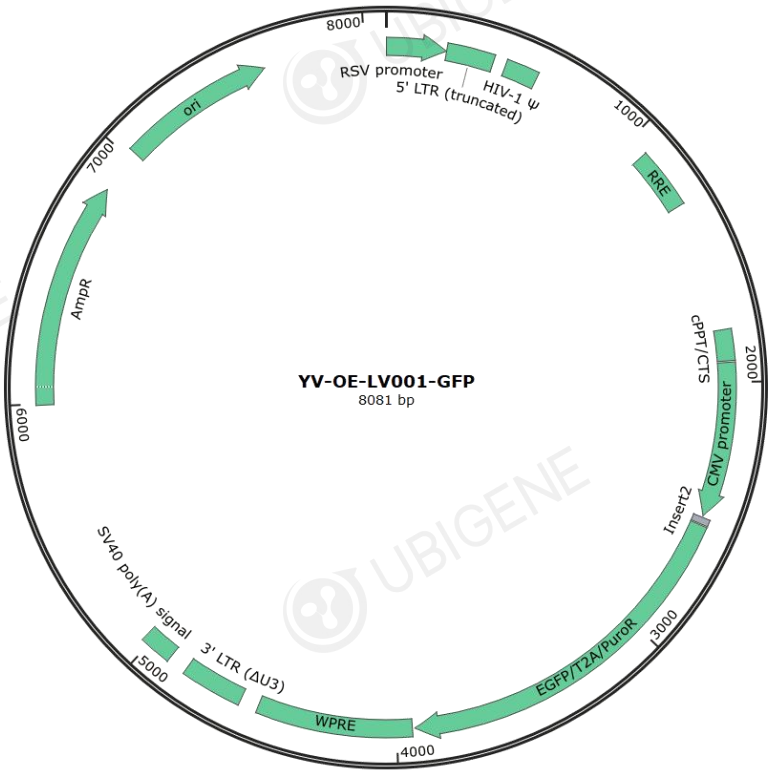
Product Use Instruction - EGFP Lentivirus

Product Info

Catalog	YV-OE-LV001-GFP	Name	EGFP Lentivirus
Quantity	Lentivirus particles: 1*10^8TU	Fluorescent & resistance	EGFP; Puro
Titer	≥1.00E+08	Storage	-80℃

Plasmid map



Storage and Handling

1) Ubigene's lentivirus product is transported with dry ice. Upon receiving, lentivirus should be stored at - 80 ℃ and avoid repeated freezing and thawing.
2) Lentivirus can be stable for at least 6 months (stored at - 80 ℃). If the storage time exceeds 6 months, it is recommended to test the virus titer again before use.

3) The infection reagent Polybrene is delivered with the lentivirus and should be stored at -20 ℃.

Transduction of Target Cells

MOI: MOI (Multiple Of Infection) is defined as as the number of infectious viral particles per cell. In other words, an MOI of 1 refers to using 1 transducing unit (TU) per cell. For different kinds of cells from different sources, the optimal MOI varies. Generally, MOI that can achieve 80% infection efficiency and will not negatively affect the cell condition would be the best MOI. For susceptible cells, the MOI is 1~10. For cells that are more difficult to be infected, MOI of 20 or higher may be required. The MOI of common cells is shown in the appendix.

Polybrene: It is an infection reagent with a common concentration of 5~8 μg/ml. Polybrene can enhance the combination of lentivirus and cell membrane by neutralizing the interaction between charges, to improve the transduction efficiency of virus. However, Polybrene is toxic to some cells, and different cells have different sensitivity to Polybrene. If necessary, several working concentrations can be set to test the toxicity of Polybrene to target cells. The concentration of Polybrene provided by Ubigene is 0.5 mg/ml. If necessary, it can be diluted with PBS or culture medium during use.

Protocol for Transducing Adherent Cells:

Day before transduction

Prepare a 12-well plate, digest the cells into single cell suspension and count the cells; Take 2*10^6 cells, evenly plate the cells into the 12-well plate, shake evenly, and place them in 37℃ incubator for culture overnight. In this case, the cells will be 30-50% confluent at the time of transduction.

Day of transduction

1) Digest the cells from 2~3 wells into single cell suspension and count the cells, then calculate the average numbers of cells from each well.

2) Take the lentivirus from the refrigerator, thaw the lentivirus on ice, and mix the virus gently by pipetting.

3) Aspirate the original medium, add 1/2 volume of fresh medium, and add Polybrene to the final concentration of 5~8 μg/mL. Calculate the required virus volume according to the virus titer T (TU/ml) and cell volume N, directly add the appropriate amount of virus as needed into the cells, shake gently, and then put it back to the incubator for further culture. The calculation formula of virus dosage is: V (μL) =1000×MOI×N/T. For example, if MOI=10, the virus titer is 4×10^8TU/mL, the cell volume is 200,000, and the added virus volume is 5μL.

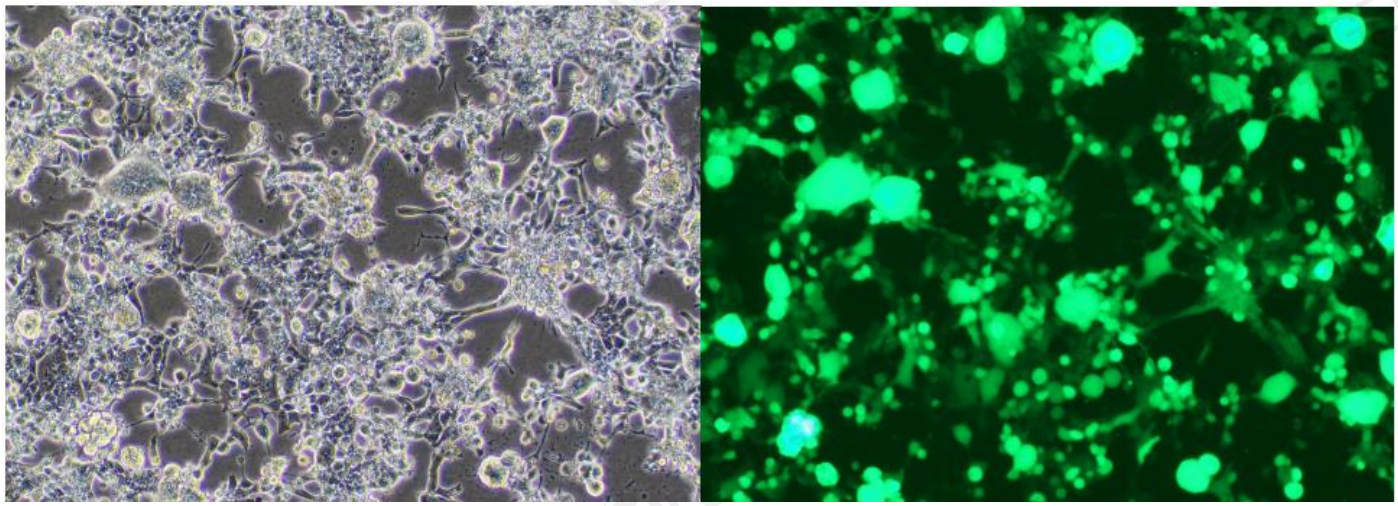
24h after lentivirus transduction

Remove the medium containing virus and Polybrene, and replace with fresh complete culture medium.

Note: Long exposure to the viral supernatant may adversely affect target cells, limit the transduction time to 6-8 hours

Lentivirus carries fluorescent gene. The fluorescent expression effect can be observed by fluorescence microscope 48~72 hours after virus infection.

The following picture was taken 48h after lentivirus (with fluorescent gene) infection of 293T cells, MOI=10.



Lentivirus carries resistance gene, antibiotic selection can be used to screen and enrich for transduced cells. To apply selection, relevant antibiotics can be added to the culture medium 48~72 hours after virus infection. The antibiotic screening experiment also needs to set one well of cells (untransduced sample) as negative control to help determine the length of antibiotic selection. Replace the antibiotics-containing culture medium every 2-3 days until the cells in the control group completely killed off.

Protocol for Transducing Suspension Cells:

1) Prepare a 12-well plate on the day of transduction, mix the cells by pipetting, and count the cells; Plate 3*10^5 cells per well and shake evenly.

2) Take the lentivirus from the refrigerator, thaw the lentivirus on ice, and mix the virus gently by pipetting.

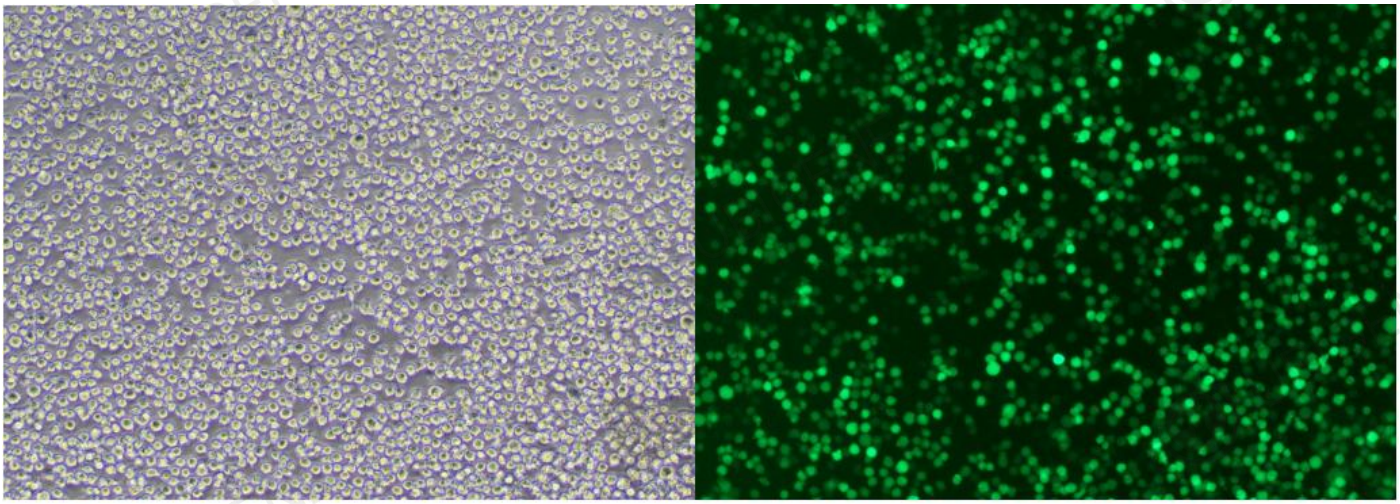
4) Add Polybrene to the final concentration of 5~8 μg/mL. Calculate the required virus volume according to the virus titer T (TU/ml) and cell volume N, directly add the appropriate amount of virus as needed into the cells, shake gently, and then put it back to the incubator for further culture. The calculation formula of virus dosage is: V（μL）=1000 × MOI × N/T. For example, if MOI=20, the virus titer is 5×10^8TU/mL, the cell volume is 500,000, and the added virus volume is 20μL.

5) 24h after lentivirus transduction, remove the culture medium containing virus and Polybrene by centrifugation, and replace with the fresh culture medium.

Note: Long exposure to the viral supernatant may adversely affect target cells, limit the transduction time to 6-8 hours

Lentivirus carries fluorescent gene. The fluorescent expression effect can be observed by fluorescence microscope 48~72 hours after virus infection.

The following picture was taken 48h after lentivirus (with fluorescent gene) infection of THP-1 cells, MOI=20.



Lentivirus carries resistance gene, antibiotic selection can be used to screen and enrich for transduced cells.

Note: In order to obtain better antibiotic screening results, it is suggested to carry out antibiotic screening preliminary experiment, to test WT cells with different concentrations of antibiotics, make a kill curve, and select a antibiotic concentration that can completely kill untransduced cells without affecting successfully transduced cells. The following table lists the antibiotic screening concentration and time for 4 common antibiotics.

Antibiotic	Puromycin	Blasticidin	Hygromycin B	G418
Common concentration	1~10 μg/mL	5~30 μg/mL	100~500 μg/mL	400~1000 μg/mL
Screening time	2~3 Days	7~10 Days	3~5 Days	4~7 Days

Safety instructions for lentivirus use

The lentivirus produced by Ubigene belongs to the third-generation lentiviral packaging system. The 3 'LTR of its genome is mutated to form the "self inactivation" (SIN), which means it will not produce new offspring viruses after the virus genome is integrated into the

cell genome. Thus, it is safe to use in vitro experiment. However, the virus still has the ability to infect human primary cells, which has potential biological hazard. Ubigene recommends that you shall wear protective equipment such as experimental clothes, masks and gloves according to the BSL-2 safety protection level, and use the biosafety cabinet for the experiment when operating the virus. The pipette tip, centrifuge tube, culture plate, waste liquid and other items that have been in contact with the virus can be sterilized (virus inactivation) by conventional sterilization procedures (121℃, 20 minutes).

FAQ

1) Lentivirus infection efficiency to target cells is low. How can I improve the lentivirus infection efficiency?

Generally, the virus infection efficiency can be improved by increasing MOI value, prolonging virus infection time and adding Polybrene. In addition, the cell condition also has a great impact on the infection efficiency. Good cell condition enables obtaining high infection efficiency. For suspension cells, they can also be infected by centrifugation.

2) After adding the virus, the cell condition becomes worse. What should I do?

It is possible that the cells are sensitive to the lentivirus or Polygene. Please reduce the MOI value of infection, stop using Polygene, observe the cell condition in time and adjust the frequency of replacing with new culture medium.