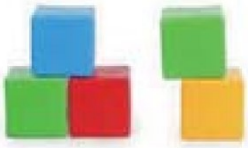
Instructions for use of HEK293 complete suspension serum-free medium



Instructions for use of HEK293 complete suspension serum-free medium

【Product name】

HEK293 Complete Suspension Serum Free Medium

【Use and description】

This medium is used for continuous culture of suspended HEK293 cells (such as 293T, 293S, 293E, 293F) and transient expression of foreign genes, especially in the process of packaging lentivirus. This product can not only support high-density expansion and continuous and stable subculture of HEK293 cells, but also show excellent transfection efficiency, highly express foreign proteins and package high titers of lentivirus.

This product is animal serum free, contains a small amount of protein components and is chemically defined.

This product does not contain glutamine. It is recommended to add it as needed during the cultivation process (the final concentration is 2 mM).

【Main components and design principles of the product】

Recombinant transferrin, recombinant human albumin, recombinant insulin, amino acids, vitamins, trace elements and other substances are adding on the basis of medium such as RPMI-1640, DMEM/F12 etc, the HEK293 cells acclimated in suspension can be directly cultured. Shear-resistant substances are added to the base to minimize cell damage during suspension culture to maintain higher viability.

【Product parameters and performance indicators】

(Note: Due to different cell sources and cell culture passages, the performance of the medium may vary. We have used the existing cell lines for multiple experimental verifications, and the following performance indicators are given.)

Supported cell densities:	4-7x10^6 cells/mL	Transfection efficiency:	50-90%
Packaging lentivirus titers:	>1.5x10^7 TU/mL	Appearance:	light yellow clear liquid
Volume:	1000 mL	Endotoxin:	<5 EU/mL
Osmotic pressure:	270-310 mOsm/kg	pH:	25℃, 6.9-7.4
Shelf life:	12 months	Storage conditions:	2-8°C, protected from light

【Instructions for use】

Note: Please take out the medium from the refrigerator before the experimental operation. Pre-warm it in a sterile bench or incubator. The medium can be aliquoted into small aliquots for equilibration. The original bottles are stored at 2-8°C.

Direct replacement (take 125 mL shake flask culture system as an example)

Normally, HEK293 cells cultured in traditional serum medium or HEK293 cells cultured in other serum-free medium can be directly transferred into Yocon HEK293 complete suspension serum-free medium (hereinafter referred to as "Yocon 293 medium"). The specific operations are as follows:

1. In order to control the cell seeding density to be about $0.5x10^6$ cells/mL, count the suspended cells, and collect the required volume of cell suspension into a 15 mL or 50 mL centrifuge tube according to the counting result.

2. Centrifuge at 170g for 3min, and discard the cell supernatant. Meanwhile, prepare a new cell shaker flask and add 25mL of Yocon 293 medium to it.

3. Add 5mL of Yocon 293 medium to the centrifuge tube and resuspend the cells, and add the cell suspension to a new shake flask.

4. The cell culture environment is 8% CO_2 and 37°C, and the rotation speed is 100 r/min. Cell growth status was monitored by cell density and viability indicators. (In the process of direct replacement, there may be a phenomenon that the counted cell density does not increase or decrease on the first day after inoculation. This phenomenon is normal, and normal growth can be restored after 2-3 days)

5. After the cells grow to the logarithmic growth phase, passage again. Proceed to normal incubation procedures.

Gradual replacement (take a 125 mL shake flask culture system as an example)

In rare cases, cells cannot grow normally after being directly replaced with Yocon 293 medium. The specific manifestations are that after passage, the cell state becomes worse, the mortality rate gradually increases, and the shape no longer presents a regular circle. In this case, it is recommended to use a step-by-step replacement method instead. The specific operations are as follows:

1. When the cells grow to the logarithmic growth phase, passage at a seeding density of $1x10^6$ cells/mL. After removing the cell culture supernatant by centrifugation, the cells were resuspended and cultured according to the mixing of 25% Yocon 293 medium with 75% original medium.

2. Repeat the previous step to allow the cells to adapt to another generation in 25% Yocon 293 medium.

3. When the cells grow to the logarithmic growth phase (about 2 days later), centrifuge the cells again, and use 50% Yocon 293 medium with 50% original medium to culture the cells.

4. When the cells grow to the logarithmic growth phase (about 2 days later), centrifuge and passage again, and use 75% Yocon 293 medium with 25% original medium to culture the cells.

5. When the cells grow to the logarithmic growth phase (about 2 days later), centrifuge and passage again, and use 90% Yocon 293 medium with 10% original medium to culture the cells.

6. When the cells grow to the logarithmic growth phase (about 2 days later), centrifuge and passage again, use 100% Yocon 293 medium to culture the cells, and enter the normal culture procedure.

Cell recovery (take 1 mL cryopreservation volume and 1x10^{7}cells/mL cryopreservation density as an example)

1. Take 20mL of Yocon 293 medium into a 125 mL shake flask 15 minutes before the cells are resuscitated, and put it in a 37°C incubator to pre-warm for later use;

2. Take 10mL of PBS equilibrated at room temperature prepared in advance and put it in a 15 mL centrifuge tube for use.

3. Take out the cryopreserved cells from the liquid nitrogen, quickly put them in a 37°C water bath, hold the cryovial with tweezers and keep shaking in the water bath until the cells are about to thaw (within 1min), and quickly transfer them to the clean bench.

4. The thawed cell suspension was added to pre-warmed PBS at room temperature.

5. Centrifuge at 170g for 3min, remove the supernatant, and resuspend the cells in the above pre-warmed Yocon 293 medium.

6. Culture in a shaker at 37°C, 8% CO_2, with a shaker speed of 100rpm.

Cell counts

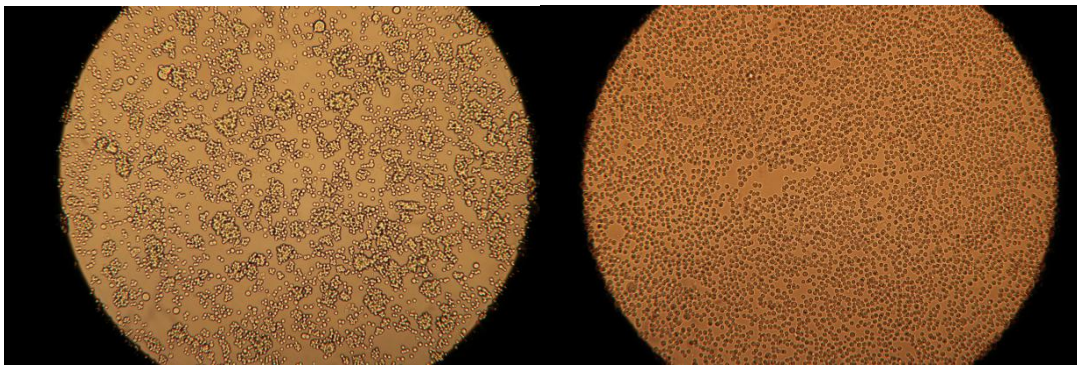
Due to the peculiar clumping growth phenomenon of suspended HEK293 cells, accurate counting results are often not obtained by using normal counting procedures. Therefore, we optimized the cell counting process by adding dispersion liquid. The specific operation process is as follows:

1. Shake up the cell culture medium in the shake flask and remove 100uL into a 1.5mL EP tube.

2. Add an equal volume of 100uL Yocon HEK293 Cell Dispersion Solution (Cat. No. NC1006), and place it in a shaking incubator at 37°C for 5-10min. If the cells clump seriously and the cell clumps are tight, the time can be appropriately extended.

3. Pipette the mixture evenly, take an appropriate volume to count with trypan blue staining or count with flow cytometer.

Note: When the cell density is high, white flocs will appear after adding HEK293 cell dispersion. This is a normal phenomenon. It can be dispersed by pipetting several times without affecting the counting result.



Before dispersion treatment ***After dispersion treatment***

Cell Growth and Serial Passaging

Take a 30mL culture system in a 125mL shake flask as an example:

1. Continuously monitor the cell density and obtain the cell growth curve.

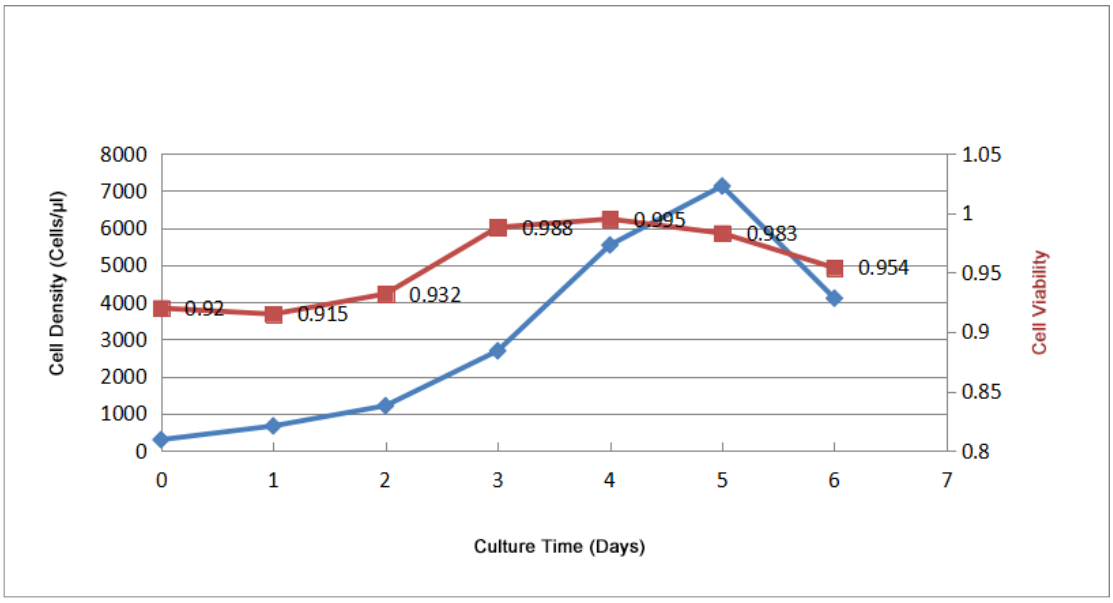
2. Sampling and counting to confirm the cell density. Cells are passaged when they are in log phase. Too high density will affect the cell state.

3. Take the required Yocon 293 culture base in a 125mL shake flask, and pre-warm it to room temperature in the incubator.

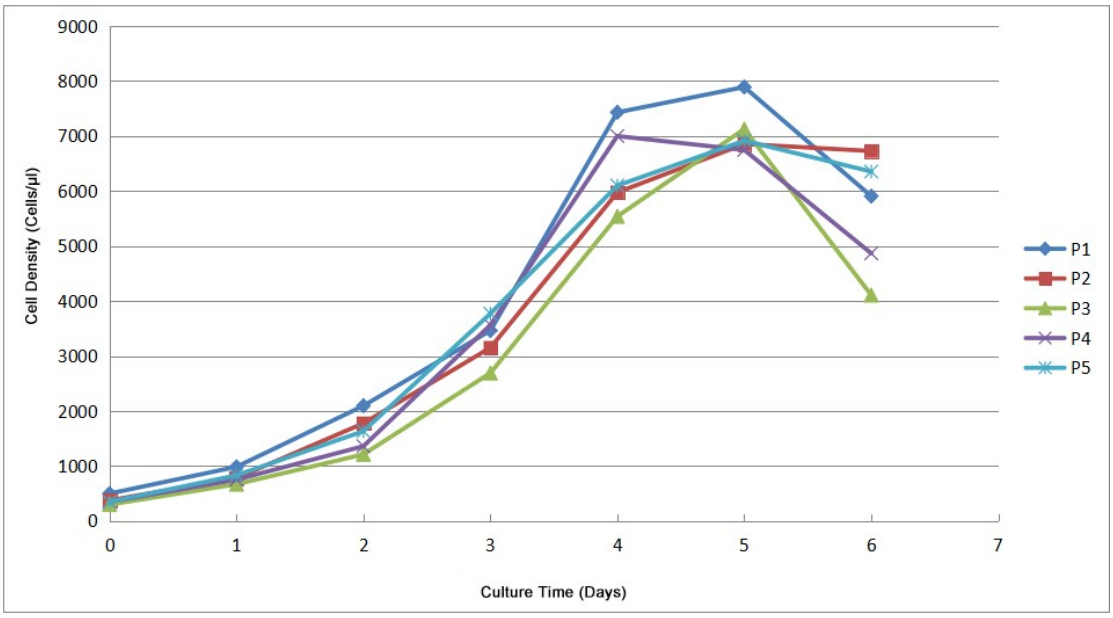
4. Take an appropriate amount of the cell culture medium to be passaged (or remove the supernatant by centrifugation) and add it to the pre-warmed Yocon 293 medium, and adjust the density to 0.3-0.5x10^6 cells/mL.

5. Inoculate at a density of 0.3x10^6 cells/mL. The cell density can reach 2-3x10^6 cells/mL on the 3rd day after inoculation. If the cells are in poor condition and the density has not reached 2x10^6 cells/mL, centrifuge the whole volume to change the medium and continue the culture.

6. If it is necessary to expand the culture, it can be directly scaled up to a 3L shake flask and 1 L culture system.



Growth and Cell Viability of 293T Cells Cultured by Yocon 293 Medium in Shake Flask



Detection of growth stability of 293T cells continuously cultured in Shake Flask by Yocon 293 medium

293T cells cultured by Yocon 293 medium in shake flasks were evenly dispersed

Take 5 L stirred reactor 1 L culture system as an example:

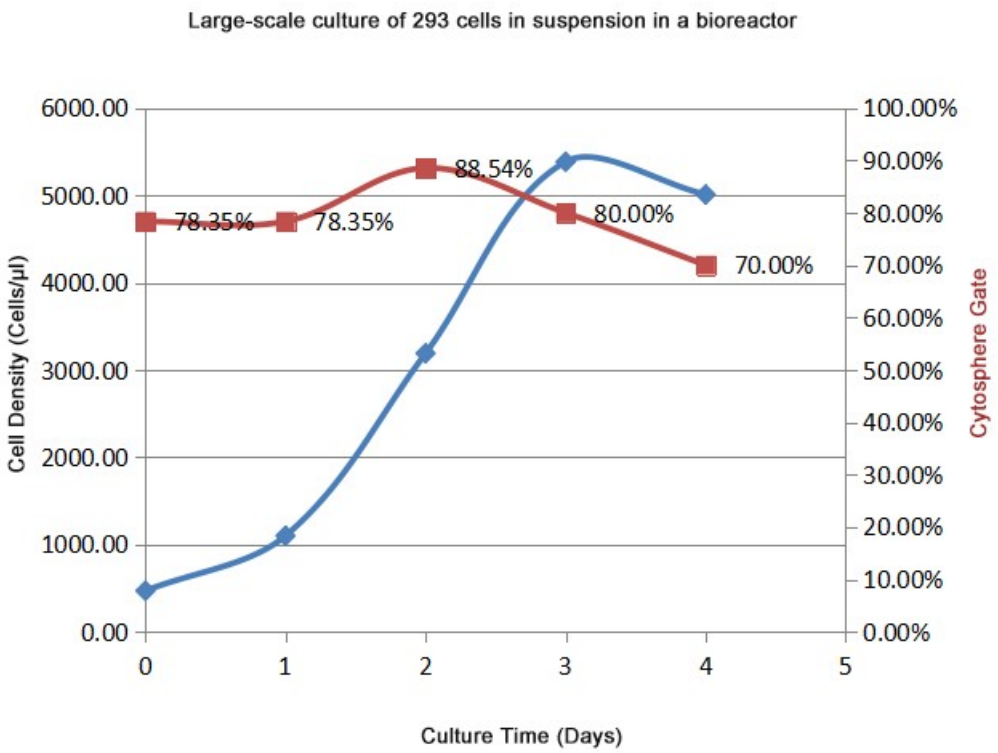
1. Prepare the reactor according to the reactor operation SOP, and perform sterility test one day before inoculation.

2. Add 1 L of fresh medium to the reactor before inoculation, set relevant parameters (temperature 37℃, PH=7.2, dissolved oxygen 40-50%, stirring speed 80-110 rpm), and prepare for inoculation after each parameter is stable.

3. Seed cell count, when the cells are in log phase can be used for inoculation.

4. If the seed cell density is $3x10^6$ cells/mL and the seeding density is $0.5x10^6$ cells/mL, 200 mL of seed cells are required.

5. Monitor the cell density every day after inoculation to obtain the cell growth curve.



Growth and cell viability of 293 cells cultured in YOCON medium in the reactor

The 293T cells cultured in the reactor Yocon 293 medium were in good condition

Lentiviral packaging

Lentiviral packaging can be performed using transfection reagents such as cationic liposomes and PEI.

Lentiviral packaging can be carried out in culture methods such as shake flasks and stirred reactors.

Take PEI transfection reagent, three-plasmid packaging system, and 125 mL shake flask 30 mL transfection system as an example:

1. Cell counting, when the density is about 2-4 x10^6 cells/mL, it can be used for lentivirus packaging.

2. Add 27 mL of fresh Yocon 293 medium to the 125 mL shake flask, and pre-warm for 15 minutes.

3. Take 30 mL of seed cells for transfection, remove the supernatant by centrifugation, resuspend in the above pre-warmed Yocon 293 medium, continue to culture and start timing.

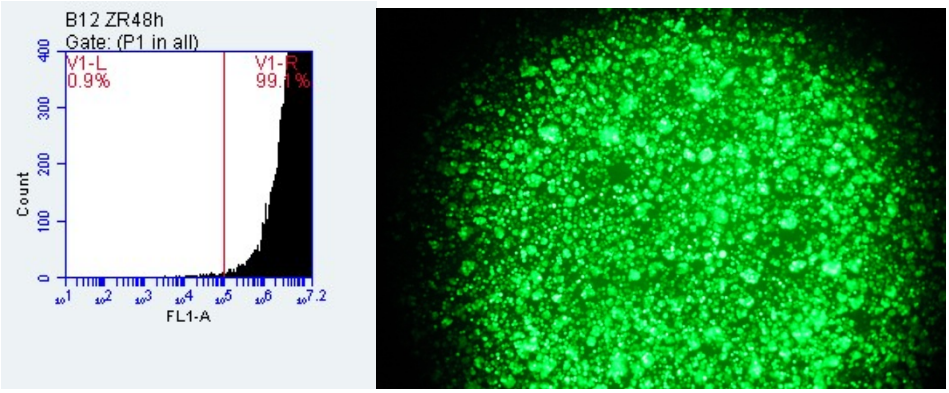
4. Prepare the DNA-PEI complexes after the cells are changed for 2 hours. Add 3mL of fresh Yocon 293 medium to a 15 mL centrifuge tube as the incubation medium, add 30ug DNA, and vortex briefly for 10s (or pipetting for 15 times). Then add 90ug PEI, briefly vortex for 10 s (or pipetting for 15 times), start timing, and incubate at room temperature for 15 min.

5. Add 3mL of the incubated DNA-PEI complex to the prepared cells, and shake the cell shaker while adding dropwise to mix.

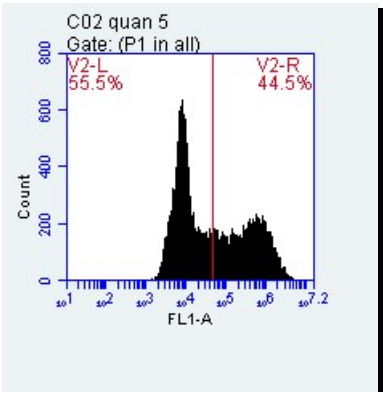
6. Immediately place the cells in a shaker at 37 °C and 8% CO_2 at a speed of 100 rpm (amplitude 50 mm).

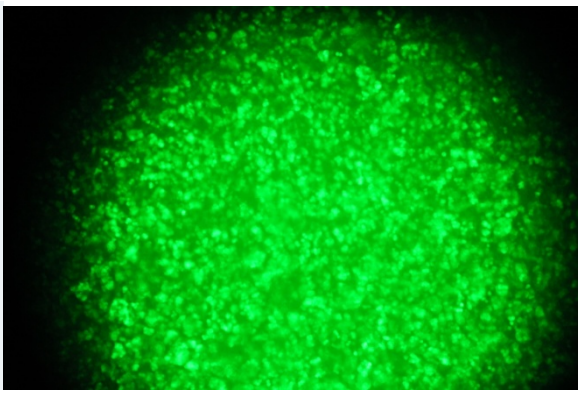
7. There is no need to change the medium or refill. 48 hours after transfection, the lentivirus-containing supernatant can be collected by centrifugation at 700 g for 10 minutes.

8. The supernatant containing lentivirus was placed in a low temperature environment of -80 ℃ for cryopreservation.



125mL shake flask 30mL transfection system: transfect after 24h the fluorescence rate is 35.6% and is 99.1% after 48h.





125mL shake flask 30mL transfection system: 5ul unconcentrated virus stock solution infects 5mL suspended 293T (2.5e5 cells/mL)

Take PEI transfection reagent, three plasmid packaging system, 5 L stirred reactor 1 L transfection system as examples:

1. Inoculate in the reactor at a density of 4-5 x10^6 cells/mL, and it can be used for transfection when the cell density reaches about 3-4 x10^6 cells/mL after about 48 hours of culture.

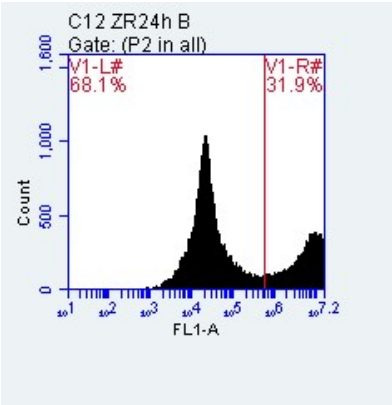
2. Discharge the excess cell culture medium in the reactor, leaving only 500mL, add 500mL of fresh Yocon 293 medium, continue the culture, and start timing.

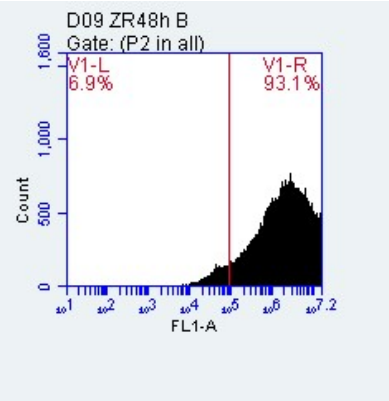
3. Prepare the DNA-PEI complex after 2 hours of cell supplementation. Add 100mL of fresh Yocon 293 medium to the 500mL inoculation flask as the incubation medium, add 1100 ug DNA, briefly vortex for 10s, and then add 3300ug PEI. Vortex for 10 s, start timing and incubate at room temperature for 15 min.

4. Add 100mL of the incubated DNA-PEI complex to the cells prepared in the above reactor.

5. There is no need to change the medium or replenish the medium. 48 hours after transfection, the lentivirus-containing supernatant can be collected by centrifugation at 700g for 10minutes.

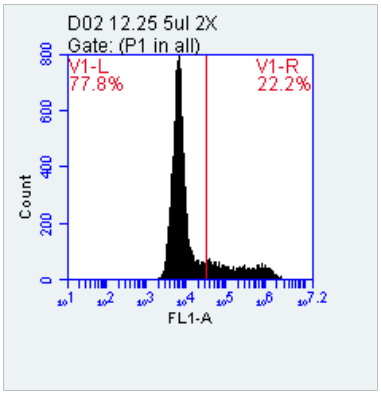
The supernatant containing lentivirus was placed in a low temperature environment of -80℃ for cryopreservation.

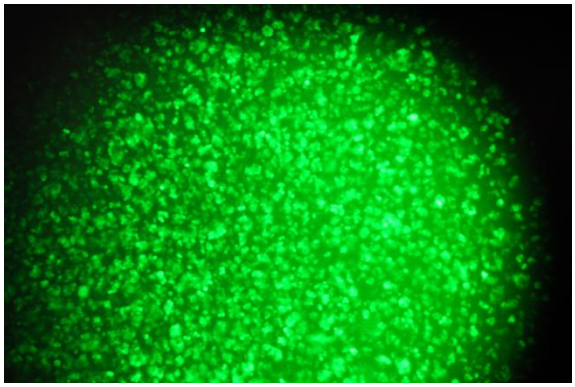




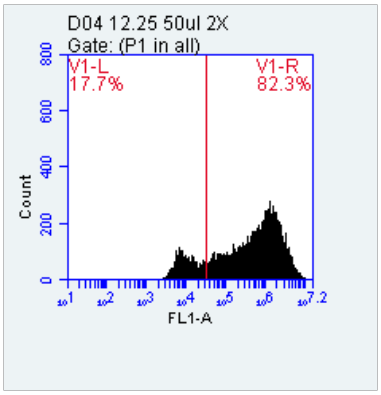
5L reactor 1L transfection system: transfect after 24h the fluorescence rate is 31.9% and is

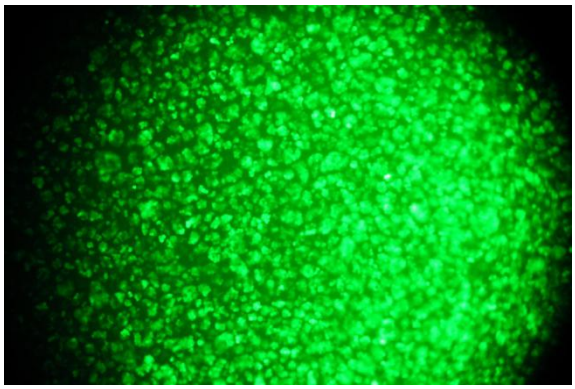
93.1% after 48h.





5L reactor 1L transfection system: 5ul unconcentrated virus stock solution infects 5mL suspended 293T (2e^5 cells/mL)





5L reactor 1L transfection system: 50ul unconcentrated virus stock solution infects 5mL suspended 293T (2e^5 cells/mL)

Cell cryopreservation

1. Sampling and counting to confirm the cell density, cells in log phase and cell viability greater than 90% should be cryopreserved.

2. Centrifuge the cell culture medium at 170g for 3min at room temperature to remove the supernatant.

3. Resuspend the cells in Yocon serum-free freezing solution (Cat. No. NC1001), adjust the freezing density to 1x10^7 cells/mL and quickly aliquot on an ice pack.

4. The subpackaged cells were immediately placed in a -80℃ refrigerator, and transferred to liquid nitrogen for long-term storage after 24h.

【Precautions】

1. This product is rich in nutrients, and there may be a small amount of precipitation at low temperature, which does not affect the use.

2. There are certain differences in the growth trend of different cell lines in this medium. It is recommended to continuously monitor the cell density to obtain the cell growth curve, so as to facilitate the selection of log-phase cells in the later stage.

3. When using this medium to package lentivirus, the transfection efficiency can reach 50-90%, but the actual lentivirus harvest is affected by the cell generation and cell status.

4. When using this medium to package lentivirus, the CO_2 content in the shaker must be adjusted to 8%.

5. If the seeded cell density is greater than 4.5 x 10^6 cells/mL, the cells that continue to be passaged are in poor condition, and it is recommended to resuscitate the cells.

6. How to increase the lentivirus titer:

1) Select cells with passages no more than 20 passages;

2) Continuous passage for 5 generations, all of which can reach a similar maximum density, confirming that the cells can be continuously and stably passaged in this medium;

3) Select log phase cells according to the growth curve;

4) Observed under the microscope, the cells are evenly dispersed, there is no cell cluster with more than 20 cells, the cells are round and full, and the refractive index is strong;

5) Passage for 3 consecutive generations after recovery, and can be used for lentivirus packaging only after the cell state is restored, and the cell viability rate is kept greater than 90%.

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