Claims

1. A method for producing lentiviral vectors from serum-free suspension cells, wherein the method comprises the following steps:

(a) providing a seed solution of packaging cells, which are packaging cells that grow in suspension, for the production of lentiviral vectors;

(b) inoculating the seed solution to a first culture solution in a culture container to obtain a first culture, wherein the first culture solution is a serum-free cell culture solution and the inoculation density is 1×10^6 to 5×10^6 cells/ml and the volume of the first culture solution is 3 to 100 L;

(c) subjecting the first culture to subculture of packaging cells under the set conditions of temperature 30°C to 38°C, dissolved oxygen 35% to 55%, CO_2 concentration 2% to 10% and pH 6.9 to 7.4, monitoring the real-time pH value of the first culture in the subculture process and controlling the concentrations of dissolved oxygen and/or CO_2 based on the real-time pH value, thereby maintaining the pH value in a range of 6.9 to 7.4;

(d) starting a liquid change operation when a liquid change trigger condition is met,

here, the liquid change trigger condition including:

(s1) real-time pH $\leq$6.9;

(s2) by adjusting the concentrations of oxygen, air, nitrogen and/or CO_2, the real-time pH value still shows a downward trend; and

(s3) subculture time $\geq$72 h;

here, the liquid change operation including: discharging the cell-free supernatant in the culture from the culture container, wherein the volume of the culture before discharge of the supernatant is Vq1, the volume of the culture after discharge of the supernatant is Vh1 and the Vq1/Vh1 ratio is 3 to 15; and then replenishing the culture solution to the culture container to form a culture of packaging cells;

(e) repeating the steps (c) and (d) n times, where n is 1, 2 or 3;

(f) starting a transfection operation when a transfection trigger condition is met

here, the transfection trigger condition including:

(t1) the total cell quantity is $0.05\sim2\times10^{11}$ cells;

(t2) the cell density is $0.5\sim5\times10^{6}$ cells/ml;

(t3) the cell viability is ≥90%; and

(t4) the total culture time is ≥72 h;

here, the transfection operation including: mixing the production plasmids and the transfection reagents for the production of the lentiviral vectors and adding them to the culture container, thereby introducing the packaging cells and forming transfected packaging cells;

(g) optionally performing liquid change after transfection;

(h) cultivating the transfected packaging cells under the set conditions of temperature 30 ℃ to 37℃, dissolved oxygen 35% to 55%, CO_2 concentration 2% to 10% and pH 6.9 to 7.4, monitoring the real-time pH value of the first culture in the subculture process and controlling the concentrations of dissolved oxygen and/or CO_2 based on the real-time pH value, thereby maintaining the pH value in a range of 6.9 to 7.4;

(i) starting harvesting and liquid change operations when a liquid change trigger condition is met,

here, the liquid change trigger condition including:

(s1) real-time pH ≤6.9;

(s2) By adjusting the concentrations of oxygen, air, nitrogen and/or CO_2, the real-time pH value still decreases; and

(s3) subculture time ≥72 h;

here, the liquid change operation including: recovering the cell-free virus-containing feed liquid in the culture, wherein the volume of the culture before recovery of the feed liquid is Vq2, the volume of the culture after recovery of the supernatant is Vh2 and the Vq2/Vh2 ratio is 3 to 15;

(j) repeating the steps (h) and (i) m times, where m is 1, 2 or 3, wherein before the repetition, the culture solution is replenished to the culture container to form a transfection cell culture;

(k) combining each of the recovered liquids to obtain combined virus-containing supernatant; and

(l) purifying the combined virus-containing supernatant to obtain purified lentiviral vectors;

here, the culture solution used in each of the above steps is a serum-free cell culture solution.

2. The method according to claim 1, wherein in the steps (c) and/or (h), culturing is conducted under a shaking condition.

3. The method according to claim 1, wherein in the step (c) and/or (h), the pH value is maintained in the range of 7.0 to 7.35.

4. The method according to claim 1, wherein in the steps (d) and/or (i), the Vq1/Vh1 ratio is 5 to 10.

5. The method according to claim 1, wherein the total time of the steps (c), (d) and (e) is 72 to 216 h.

6. The method according to claim 1, wherein in the step (f), the multi-plasmid transfection used includes three-plasmid transfection and four-plasmid transfection.

7. The method according to claim 1, wherein the culture container is a disposable culture container.

8. The method according to claim 1, wherein the total number of viruses contained in the combined virus-containing supernatant is 1×10^{12} Tu.

9. The method according to claim 1, wherein the packaging cells are human embryonic renal epithelial cells.

10. The method according to claim 1, wherein in the step (1), the purification includes: ultrafiltration and chromatography.