Product Instruction

293 Cell Serum-free Medium

Product Type: Celer-S101S

Product Description

Celer-S101S 293 cell serum-free medium is a customized medium with clear chemical composition, protein-free and non animal-derived components, which is developed by Shanghai BioEngine Sci-Tech Co., Ltd. This medium is suitable for suspension culture of 293 cells (human embryonic kidney cells) and expression of products based on transient transfection. Its characteristics are:

- Completely serum-free
- Does not contain any components of animal origin
- No need to add serum or plasma
- Clear chemical composition
- Applicable to virus packaging based on transient process

Product Formula

The intellectual property rights of Celer-S101S 293 cell serum-free medium formula are owned by Shanghai BioEngine Sci-Tech Co., Ltd. For additional information, please contact our technical support department.

Product Preservation

- Store in a dark environment at 2-8℃.
- This product is vulnerable to water damage. Please use immediately after opening. If it needs to be stored, please sealed by heat sealing and sealing clips, avoiding damp and being ineffective.
- Do not recommend to use, when the product is beyond expiration date.

Product Failure

This product is light yellow or similar color powder, with good fluidity. The storage life is two

years when sealed. The solution prepared according to instruction is a clear and transparent pink liquid. The product may be considered to be ineffective, if the following conditions occur. In these conditions, please contact the technical support department/after-sales service department of Shanghai BioEngine Sci-Tech Co., Ltd. in time.

- Agglomerates that are not easily crushed appear in the dry powder.
- Deliquescence in the dry powder.
- Visible insoluble matter after preparation.

Preparation of the medium

Do the preparation of Celer-S101S 293 cell serum-free medium as per the one Table 1 shows.

Component	Concentration
Dry powder of the medium	23.00 g/L
Sodium bicarbonate	2.30 g/L

Table 1 Preparation of Celer-S101S 293 cell serum-free medium

(1) Weigh 100% water of the final medium preparation volume into the medium preparation container. When preparing, ultrapure water or water for injection and above standard water should be used, and the water temperature should be controlled at 20-30 °C.

(2) Turn on the mixing system of the medium preparation container, stir thoroughly, and avoid the generation of air bubbles during stirring.

(3) Accurately weigh 23.00 g/L of dry powder, add them into the preparation container near the liquid surface or use special equipment such as homogenizer, and stir thoroughly for 20 min.

(4) Slowly add, dropwise, 5 mol/L sodium hydroxide solution to the solution prepared in (4), adjust its pH value to 6.0-6.5, and stir thoroughly for 20 min.

(5) Accurately weigh 2.30 g/L sodium bicarbonate powder, and add them into the preparation container near the liquid surface or use special equipment such as homogenizer, and stir thoroughly for 20 min.

(6) Use 1 mol/L hydrochloric acid solution to adjust the pH value of the medium to 7.0-7.4 (If necessary).

(7) It is recommended to use a pulse pump or compressed air (3-15 psi) to sterile filter the medium solution through a sterile filter membrane with 0.22 µm pore size.

(8) Store in a dark environment at 2-8°C, and the expiration date is one month.

Medium usage

Cryopreservation

Select the cells that are in good condition in the logarithmic growth phase for cryopreservation. The cryopreservation density is 2-3×10^7 cells/ml/tube. The ratio of cryopreservation solution is 93% fresh medium + 7% DMSO. Centrifuge the cells under the speed of 1,000 r/min for 5 min, discard the supernatant, resuspend the mixed freezing solution, and dispense the cells into freezing tube, 1 ml/vial. Then, place them in a programmed cooling box at -80℃ overnight, and transfer to liquid nitrogen save

Recovery

Rotate the freezing tubes in the same direction in a 37°C water bath to quickly melt the cryopreservation solution, and take them out to a clean bench when only small ice crystals remain. Quickly add the cryopreservation solution to 10 ml of medium, and centrifuge them under the speed of 1,000 r/min for 5 min, discard the supernatant and wash away the DMSO. Use 20-30 ml of medium to resuspend the cells, and control the seeding density at 0.8-1.2×10^6 cells/ml.

Subculture

Use Celer-S101S 293 cell serum-free medium for dilution and subculture. The recommended seeding density is 1.0×10^6 cells/ml, and subculture should be carried out every 48 hours.

Cell transfection

Taking a 125 ml shake flask as an example, the working volume is 30 ml:

- Take the cells in the exponential growth phase and inoculate them in a sterile ventilated shake flask at an initial density of about 1.0×10^6 cells/ml. After 48 hours, the density will be 3-4×10^6 cells/ml, and the viability rate should be greater than 90%;
- Before transfection, dilute the cell density to about 1-2×10^6 cells/ml with fresh medium and wait for later use;
- The total amount of plasmid required per 1×10^6 cells is 1 µg, and PEI is 2-3 µg;
- The total incubation volume should be 3%-5% of the working volume.
- Dilute the plasmid to 500 µl with serum-free DMEM, mix gently and incubate for 5-10 min;
- Dilute PEI to 500 µl with serum-free DMEM, mix gently and incubate for 5-10 min;
- Add DNA diluent to PEI diluent, mix gently and incubate for 10 min;
- During transfection, use a pipette to aspirate the dilution of the plasmid-PEI complex, add it into the cell culture medium, shake gently and put into the incubator and continue to cultivate until harvest.