Product Instruction

293 Cell Serum-free Medium

Product Type: Celer-S201S

Product Description

Celer-S201S is developed by Shanghai BioEngine Sci-Tech Co., Ltd., which is chemically defined, protein-free and animal origin component-free 293 cell serum-free medium. It is suitable for suspension culture of 293 cells (human embryonic kidney cells) and expression of products based on transient transfection.

Storage

- 2–8°C;
- Store dark and dry

Shelf life

- Shelf Life is 24 months from Date of Manufacture.

Preparation Instructions

- According to the formula shown in the table to prepare

Formula	Concentration
Dry powder Medium	23.54 g/L
$NaHCO_3$	2.2 g/L

- Measure 100% final required volume of deionized or distilled water into a medium preparation vessel, and keep water in room temperature (20°C to 30°C).
- Start mixing gently and avoid the generation of bubbles.
- Add 23.54 g/L of Celer-S201S dry powder medium slowly to the vessel, mix for at least 20 minutes, the solution will be cloudy but without any clumps or dry powder residues.
- Adjust the pH of 6.0-6.5 with 5 N NaOH solution slowly, Mix for at least 20 minutes. The

solution will be clear.

- Add 2.2 g/L sodium bicarbonate (NaHCO3) into the vessel, mix for at least 20 minutes, the pH will be 7.0-7.4. Adjust the pH by drop-wise addition of 5 N NaOH or HCl if necessary.
- Sterilize by membrane filtration, Store product at 2°C to 8°C (protected from light) up to 6 months.

Application

Subculture

- Determine viable cell density before cell passage, we recommend cell viability should be above 90% for passage.
- Take the cells in exponential growth period, inoculate them in a shaker flask at an initial density of about 1.0×10^6 cells/ml
- Incubate the cells in a 37℃ incubator with 5% CO_2 on an orbital shaker with 110-130 rpm.
- Passage every 48 hours.

Cryopreservation

- Choose cells cultured in good growth profile for Cryopreservation, Freeze cells at a final density of 2.0×10^7-3.0×10^7 viable cells/ml.
- Use a freezing medium composed of 93% fresh Celer-S201 medium and 7% DMSO.
- Centrifuge the cells at 175 g for 5min, then discard the supernatant, and resuspend with the mixed cryopreservation solution. 1 ml/vial is sub packaged into a freezing tube.
- Put freezing tubes in a program cooling box at -80 ℃ overnight, and transfer it to liquid nitrogen for storage.

Thaw

- Rapidly thaw (1–2 minutes) a frozen vial cells in a 37°C water bath.
- Add 10 ml fresh medium into the centrifuge tube, transfer the entire cells of the freezing tube into the centrifuge tube, centrifuge 175g for 5min, and wash off DMSO.
- Use fresh medium to resuspend cells in a shake flask at an inoculation density of $0.8\text{-}1.1\times10^6$ cells/ml.

Transfection

- Take the cells in exponential growth period, inoculate them in a shaker flask at an initial

density of about 1.0×10^6 cells/ml, and culture for 2-3 Days.

- Dilute the cell density to about 2×10^6 cells/ml with fresh medium before transfection.
- The amount of pDNA is 1μg per 1×10^6 cells, and the ratio of PEI to pDNA is4:1-2:1.

  Note: The amount of pDNA and the PEI/pDNA ratio can be optimized.
- The total incubation volume is 3%-5% of the working volume.
- Dilute pDNA to 1/2 of total incubation volume with fresh DMEM, mix gently and incubation for 5-10min at room temperature.
- Dilute PEI to 1/2 of total incubation volume with fresh DMEM, mix gently and incubation for 5-10min at room temperature.
- Add the diluted pDNA to the diluted PEI, mix gently and incubation for 10min at room temperature.
- Add the pDNA-PEI complex to the cell suspension prepared in Step 2 and shake gently.
- Incubate the cell suspension in a 37℃ incubator with 5% CO_2, and harvest on Day 7 or while viability is less than 50%.