Product Instruction

293 Cell Serum-free Medium

Product Type: Celer-S101

Product Description

Celer-S101 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 production of recombinant virus vector (AAV and LV) based on transient transfection.

Storage

- 2–8°C;
- Store dark and dry

Shelf life

- Shelf Life is 6 months from Date of Manufacture.

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 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 0.8-1.1×10^6 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 is 4:1-2:1.

  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 5-10 min 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 post transfection of 48 h or 72 h.