Celkey® CD ST 258

Version 1.1

Celkey® CD ST 258 medium is a serum free chemically defined medium specially for ST suspension cells, it is used to culture, virus proliferation of ST suspension cells. It does not contain animal-derived components, proteins, hydrolysates and other undefined components. which can support SFV (Swine Fever Virus), PPV (Porcine Parvovirus), PRV (Pseudorabies Virus) and PEDV (Porcine Epidemic Diarrhea Virus) related veterinary live and inactive vaccines large-scale industrial production.

Product	Catalog No.	Form	Package Sizes
CD ST 258	10604-258	Dry powder	2 L, 10 L, 100 L, Customized

Safety Warning

Read the Material Safety Data Sheets (MSDS) and follow handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Intended Use

For research and further manufacturing use only.

Component information

With 8.0 g/L glucose, 2.0 mM L-glutamine, sodium bicarbonate, Pluronic F-68.
Without phenol red.

Storage and Stability

The dry powder medium should be stored at 2–8°C and protected from light. The product is stable for 24 mouths when unopened and stored properly. This product is very hygroscopic and should be kept in a dry environment away from moisture.

Prepare Liquid Medium from Dry Powder

1. Fill the mixing container with purified water (35–40°C) at 80%–90% of the final volume.
2. Slowly add 28.64 g/L of dry powder medium with gentle stirring. Mix for 30 minutes.
3. Add 0.50 mL/L of TE001 (99053-13006, shipped with CD ST 258 medium), Mix for 10 minutes.
4. Adjust the pH to 6.8–7.0 using 1 M–5 M NaOH or HCl. The pH may rise 0.1 to 0.2 after sterile filtration.
5. Adjust the final volume with purified water. Mix for 10 minutes.
6. Filter the media using a membrane filter with 0.22 μm pore size immediately.

Note: Avoid repeated prewarming medium. The pH of the medium should be in the range of 7.0–7.4 before use.

Culture Conditions

Medium: CD ST 258
Cell line: ST cells

Inoculation density: 0.6–0.8 × 10^6 cells/mL
Culture type: Suspension
Culture vessels: TPP tubes, shaker flasks, or 3 L bioreactor
Recommended parameters for different culture vessels:

TPP Tubes	
Items	**Recommended Parameters**
50 mL TPP tubes	Culture Volume: 10–30 mL
Shaking Speed	200 rpm @50mm orbital throw shaker
Culture Temperature	37°C
CO_2 Concentration	5%
Relative Humidity	80% RH
125 mL Shaker Flask	Culture Volume: 10–40 mL
250 mL Shaker Flask	Culture Volume: 40–80 mL
500 mL Shaker Flask	Culture Volume: 100–150 mL
1000 mL Shaker Flask	Culture Volume: 200–300 mL
Shaking Speed	125–150 rpm @25 mm orbital throw shaker 90–120 rpm @50 mm orbital throw shaker
Culture Temperature	37°C
CO_2 Concentration	5%
Relative Humidity	80% RH

3 L Bioreactor	
Items	**Recommended Parameters**
Shaking Speed	150–200 rpm Adjust according to the ratio of diameter to blade of reactor of different manufacturers
Culture Temperature	37°C
CO_2 Sparger	0.01 VVM
Air Sparger	0.01 VVM
O_2 Sparger	The initial setting is 0.02 VVM It should be upregulated according to the increase of viable cell density.
pH	7.1 ± 0.2
DO	30%–50%
Note: An additional 1.0 g/L Pluronic F-68 is recommended to protect cells from shear force during bioreactor culture.	

Recover Cells

The cryopreserved volume is 1.0 mL/ tube, the cryopreserved density is 3.0 × 10^7 cells/mL, and the cells are recovered into 125 mL shaker flask for suspension culture as an example.

1. Remove the cryopreserved tube from the liquid nitrogen, rapid thaw (<1 minute) the frozen cells in 37°C water bath, avoid immersing the lid of cryopreserved tube into the water.
2. Transfer all cell fluid into a sterile centrifuge tube with 30 mL prewarmed CD ST 258 medium, centrifuge the cells at 1000 rpm (about 233 × *g*) for 5 minutes, aspirate and discard the supernatant, then resuspend cells with fresh prewarmed CD ST 258 medium and transfer them into sterile 125 mL shaker flask. Determine viable cell density (× 10^6 cells/mL) and viability (%) using cell counter equipment, adjust the cell density to 0.6–0.8 × 10^6 cells/mL.
3. Culture the cells referring to the "Culture Conditions". 24 ± 4 hours after recover, sample cell fluid to determine viable cell density (× 10^6 cells/mL) and viability (%) using cell counter equipment.
4. Passage the cells every 72 ± 4 hour, and passage method should refer to the steps of "Cell Passage and Scale-up". It is recommended other experiments carried out after 3–5 continuous passages after cells growth stable.

Cell Passage and scale-up

The cells which are cryopreserved in CD ST 258 medium and after successful recover or have been adapted to CD ST 258 medium can be conducted continuous passage directly, and scale-up the cells according to the needs of subsequent experiments. The steps of cell passage and scale-up are as follows:

1. Taking sample of cells fluid to determine viable cell density (× 10^6 cells/mL) and viability (%) using cell counter equipment. Calculate required volume of CD ST 258 medium and the cell fluid according to the seeding density of 0.6–0.8 × 10^6 cells/mL.
2. required volume of cells fluid according to calculate result, resuspend cells with prewarmed CD ST 258 medium.
3. Culture cells referring to the "Culture Conditions". It is recommended that viable cell density (× 10^6 cells/mL) and viability (%) should be determined every day during the first passage to the next passage. Before passage, if the cell density <3.0 × 10^6 cells/mL or the viability <90%, centrifuge the cells at 1000 rpm (about 233 × *g*) for 5 minutes, aspirate and discard the supernatant, then resuspend cells with 100% prewarmed fresh CD ST 258 medium according to the seeding density 0.6–0.8 × 10^6 cells/mL, and transfer them into sterile vessel. Culture cells referring to the "Culture Conditions" continue to culture.
4. Passage the cells every 72 ± 4 hour. It is recommended other experiments carried out after 3–5 continuous passages after cells growth stable.

Adapt Cells to CD ST 258 Medium

Adaptation is recommended if ST cells are transferred from a different medium or culture type into CD ST 258 medium, there are direct adaptation and sequential adaptation.

Direct Adaptation

Cells in good condition are conducive to direct adaptation. It is recommended to follow the previous routine culture regimen. Healthy ST cells in other medium, centrifuged the cells at 1000 rpm (about 233 × *g*) for 5 minutes, aspirate and discard the supernatant, then directly adaptive in CD ST 258 medium. The cells passage is carried out according to the steps of "Cell Passage and Scale-up". If the cell state is not good during direct adaptation, sequential adaptation is recommended.

Sequential Adaptation

Transfer ST cells grown in other media to CD ST 258 medium carried out sequential adaptation as follows:

1. The process of sequential adaptation needs to be carried out step by step in different proportions of the original medium mixed with CD ST 258 medium. The mix proportions of the original medium (including the original medium and reagent) and CD ST 258 medium ratio are 90 : 10, 75 : 25, 50 : 50, 10 : 90, 0 : 100, at least 2–3 passages should be carried for each mixed medium, this process could be adjust according to cell growth conditions.
2. On the day of passage, determine viable cell density (× 10^6 cells/mL) and viability (%), calculate the required volume of culture medium and cell fluid according to the seeding density of 0.6-0.8 × 10^6 cells/mL and seed the cells into a new sterile vessel.

3. The passage of sequential adaptation process is carried out according to the steps of “Cell Passage and Scale-up”. The cells culture is referring to the “Culture Conditions”.
4. When ST cells were completely transferred to 100% CD ST 258 medium, cells passage referring to “Cell Passage and Scale-up”. It is recommended other experiments carried out after 3–5 continuous passages after cells growth stable.

Cryopreserve Cells

Cryopreservation is recommended for cells in mid-log phase of growth with viability >90%.

1. Put the freezing container with fresh isopropanol, the cryopreserved tubes and other required materials into 2–8°C for pre-cooling for 24 hours or −20°C for pre-cooling for 2–4 hours in advance.
2. Determine viable cell density (× 10^6 cells/mL) and viability (%) using cell counter equipment, The cryopreserved volume is 1.0 mL/ tube, the cryopreserved density is 3.0 × 10^7 cells/mL cells/mL to calculate and prepare related liquids according to the following table:

Items	**Formula**
Required volume of cryopreservation solution (mL)	20% DMSO (mL) + 80% CD ST 258 Medium (mL)
Required volume of cell resuspension fluid (mL)	Adaptive culture medium (mL)
Required total number of cells (× 10^7 cells)	Cryopreserved density (× 10^7 cells/mL) × Required number of cryopreserved tubes (tubes) × Cryopreserved volume/ tubes (mL)
Required volume of cell fluid (mL)	Required total number of cells (× 10^7 cells) ÷ The determined viable cell density (× 10^6 cells/mL)
Required volume of cryopreserved cell suspension (mL)	Cryopreserved volume/ tubes (mL) × Required number of cryopreserved tubes (tubes) Required volume of cell suspension (mL) + Required volume of cryopreservation solution (mL)
Note： Volume of cryopreservation solution (mL) = Volume of cell resuspension fluid (mL). Cryopreservation solution should be prepare as needed and put it on ice or pre-cool or at 2–8°C. It is recommended that the number of cryopreserved tubes are 1–2 more than the required number. The final cryopreserved cell suspension contained 10% DMSO after cryopreservation fluid is added into cell suspension drop by drop.	

3. Sample the cell fluid according to the calculation results, centrifuge the cells at 800–1150 rpm (about 150–300 × *g*) for 5 minutes, transfer the supernatant to a suitable sterile container for storage (This is adaptive culture medium), then gently pat the bottom of the centrifuge tube to make the cell precipitation evenly dispersed. Add the required volume of adaptive culture medium for resuspension according to the calculation results and prepare the cell suspension.
4. Add the pre-cooled cryopreservation solution drop by drop into the cell suspension to prepare the cryopreserved cell suspension, and gently shake while adding to ensure the uniformity of cells. This step needs to be carried out in the ice bath.
5. Place the pre-cooled cryopreservation tubes on the ice, and the cryopreserved cell suspension are divided into 1 mL of volume for each tube. During the loading period, the cryopreservation cell suspensions were mixed with light shaking repeatedly.
6. After the separation, transfer the cryopreserved tubes to the pre-cooled freezing container, store it in the −80°C refrigerator for at least 24 hours, and transfer it to the liquid nitrogen tank for long-term storage.