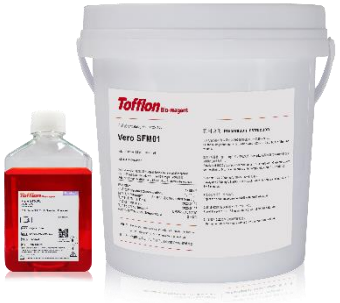
Version 2，2023

The User's Manual—Vero SFM01

Basic Information Introduction

Product Introduction

Vero SFM01 is a Serum-Free basal medium, without protein and any animal-derived components, developed specifically for Vero cell lines, it is suitable for Vero cell adherent static culture and microcarrier suspension large-scale culture, supporting high-density cell growth and viability maintenance, it can be used in the development and production process of influenza vaccine and other related products.



Application Scope

Vero SFM01 can be used in the process of Vero cell thawing, cell passage, adherent static culture, and microcarrier suspension large-scale culture. The basic medium is suitable for scientific research and the production of large-scale biological products based on cell culture, but cannot be directly used in the human body or used as a medication.

Shipping, Storage and Validity Period

Product	Catalog No.	Storage	Shipping	Validity Period
Vero SFM01	LQ25, Liquid	2℃ ~ 8℃, Protect from light	2℃ ~ 8℃, Protect from light	12 months
Vero SFM01	DP25, Powder	2℃ ~ 8℃, Protect from light	2℃ ~ 8℃, Protect from light	24 months

Protocol for Hydration of Powder Medium

1. Fill the mixing container with purified water (25 ~ 30℃) at 90% of the final volume.
2. Slowly add 15.00 g/L of powder medium and 584 mg/L of L-Glutamine with gentle stirring. Mix for 40 minutes until completely clear (no heating).
3. Slowly add 2200 mg/L $NaHCO_3$ to the solution with gentle stirring. Mix for 3 ~ 5 minutes.
4. Adjust the solution volume to 100% with purified water.
5. Adjust the pH to 7.1 ~ 7.3 using 1M HCl or 1M NaOH solution.
6. Filter immediately the media with a 0.22 μm membrane filter.

Quality Index of Powder and Liquid Media

Product Index	Vero SFM01 (LQ25), Liquid	Vero SFM01 (DP25), Powder
Appearance	Red, clear liquid	Off- pink powder
pH	7.1 ~ 7.5	7.1 ~ 7.3 (pre-filter)
Osmolality (mOsmol/kg)	270 ~ 320	270 ~ 320
Solubility	--	Dissolve well according to the protocol for hydration of powder medium
Endotoxin (EU/mL)	< 3	< 3
Sterility	Negative	--
Bioburden	--	Aerobic bacteria: < 200 CFU/g Molds and yeasts: < 50 CFU/g

Reference Cell Culture Protocol

Culture Conditions

Parameter		Value
Culture volume	T25 cell culture flask	5 ~ 10 mL
	T75 cell culture flask	15 ~ 25 mL
	T175 cell culture flask	40 ~ 55 mL
	1-layer Cell Factory	130 ~ 200 mL
	2-layer Cell Factory	260 ~ 400 mL
	10-layer Cell Factory	1300 ~ 2000 mL
Culture environment	Seeding density	2 ~ 4×10^4 cells/cm^2
	Incubation temperature	37℃
	Incubation CO_2 concentration	5%
	Incubation relative humidity	> 80% RH

Cell Thawing

1. Pre-warm the medium (Vero SFM01) in 37℃ water bath.

2. Spray the outside of the medium bottle with 75% alcohol and place the bottle into the bio-safety cabinet.

3. Thaw one vial at a time in 37℃ water bath. Gently agitate the vial within 1 minute until the ice in the vial melting.

4. Pipet the contents from the vial gently into a centrifuge tube containing 10 mL of pre-warmed medium (Vero SFM01).

5. Centrifuge 150 g to 300 g for 5 minutes. Discard the supernatant and re-suspend cells in 10 ~ 30 mL fresh pre-warmed medium (Vero SFM01), then adjust the cell density to 2 ~ 4×10^4 cells/cm^2.

6. Sample 0.5 mL of cell suspension and analyze the viable cell density ($\times10^6$ cells/mL) and viability (%) of the sample using cell counter.

7. If the cell viability > 90%, incubate cells in the specified condition (refer to "culture conditions" table).

Cell Passage

Take the T75 flask as an example:

1. Pre-warm the medium (Vero SFM01) in 37℃ water bath for 20 ~ 30 min.

2. Cells with cell viability ≥ 90%, and in the middle of logarithmic growth phase were selected for passage.

3. Aspirate medium from cell monolayer and rinse flask with 7 ~ 10 mL pre-warmed DPBS. Aspirate DPBS.

4. Add 1 mL of Recombinant Trypsin Solution (PDE RecTrypsin-LQ02 (1×), PDE-LQ02/ PDE RecTrypsin-LQ03 (10×), PDE-LQ03) to flask, infiltrate all culture surfaces, and digest for 3 ~ 5 minutes at room temperature.

5. When the cells fall off from the bottle wall, pat the bottle wall, add the preheated mixture (containing 1 mL of 0.5% trypsin inhibitor and 9 mL of Vero SFM01 medium, a total of 10mL) to pipette and disperse the cells, collect the cells, and centrifuge the cell suspension at room temperature 300 g for 5 ~ 10 minutes.

6. Discard the supernatant and resuspend the cell pellet with Vero SFM01 medium.

7. According to the seed density of 2 ~ 4×10^4 cells/cm^2, calculate the amount of total number of seed cells.

8. Seed cells at 2 ~ 4×10^4 cells/cm^2 in the T75 flask and add a certain volume of pre-warmed fresh medium.

9. Passage cells with fresh medium according to the above steps every 3 to 5 days.

Adaptation

1. For cells can direct adapt, transfer cells suspension cultures into Vero SFM01 directly, and the seed cell density refer to the cell passage procedure.

2. Cell passage until cell expression steadily.

3. The recommended cell passage seeding density was 2 ~ 4×10^4 cells/cm^2, and the cells were subcultured every 4 to 6 days. Generally, three passages of cells could be completely adapted.

Cell Cryopreservation

1. Prepare cells, harvesting in mid-log phase of growth with viability > 90%.

2. Sample and cell counting, calculate the required volume of cell freeze solution to give a final density of 1×10^7 cells/mL.

3. Prepare the cell freeze solution: 90% Vero SFM01 + 10% DMSO, refrigerate at 4℃.

4. Prepare the cell suspension according to the cell digestion procedure.

5. Centrifuge 300 g for 5 minutes, discard the supernatant and re-suspend cells with the cell freeze solution.

6. Immediately dispense aliquots of cells suspension into cryovials according to the specific needs of the project.

7. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1℃ decrease per minute).

8. Transfer to liquid nitrogen tank for storage.