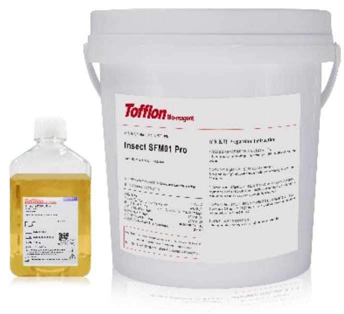
Version 2，2023

The User's Manual—Insect SFM01 Pro Pro

Basic Information Introduction

Product Introduction



Insect SFM01 Pro Pro is a Serum-Free medium, without protein, protein hydrolysate and any animal-derived components, developed specifically for Insect cell lines, combined with Insect TE02 additive, it suitable for high-density cell suspension expansion and baculovirus transfection expression of Insect cells, supporting high-density cell growth and viability maintenance, it can be used in the development and production process of related products.

Application Scope

Insect SFM01 Pro Pro combined with Insect TE02 additive, can be used in the process of Insect cell fed-batch cultivation and baculovirus transfection expression. 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
Insect SFM01 Pro Pro	LQ20, Liquid	2℃ ~ 8℃, Protect from light	2℃ ~ 8℃, Protect from light	12 months
Insect SFM01 Pro Pro	DP20, 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 (20 ~ 30℃) at 90% of the final volume.
2. Slowly add 43.40 g/L of powder medium with gentle stirring. Mix for 20 ~ 30 minutes.
3. Adjust the pH to 6.0 ~ 6.2 with 5 mol/L NaOH, and continue to stir for more than 20 minutes until the powder is completely dissolved.
4. Slowly add 0.5 mL/L of TE02 and fill with purified water to the final volume. Adjust the pH to 6.0 ~ 6.2.
5. Filter immediately the media with a 0.22 μm membrane filter.

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Quality Index of Powder and Liquid Media

Product Index	Insect SFM01 Pro Pro (LQ20), Liquid	Insect SFM01 Pro Pro (DP20), Powder
Appearance	Yellow, clear liquid	Light yellow or similar color powder
pH	5.9 ~ 6.4	6.0 ~ 6.2 (pre-filter)
Osmolality (mOsmol/kg)	350 ~ 390	350 ~ 420
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: < 200 CFU/g

Reference Cell Culture Protocol

Culture Conditions

Parameter		Value
Culture volume	50 mL TPP Tube	10 ~ 30 mL
	125 mL Shake flask	15 ~ 40 mL
	250 mL Shake flask	40 ~ 80 mL
	500 mL Shake flask	100 ~ 200 mL
	1000 mL Shake flask	200 ~ 300 mL
Shaking speed	TPP Tube	50mm amplitude：200 rpm
	Shake flask	25mm amplitude：150 rpm
	Shake flask	50mm amplitude：90 ~ 120 rpm
Culture environment	Seeding density	1.0×10^6 cells/mL
	Incubation temperature	27℃
	Incubation CO_2 concentration	Air content
	Incubation relative humidity	> 80% RH

Cell Thawing

1. Pre-warm the medium (Insect SFM01 Pro) in 27℃ 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 (Insect SFM01 Pro).

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 (Insect SFM01 Pro), then adjust the cell density to 1.0×10^6 cells/mL.

6. Sample 0.5mL 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 > 85%, incubate cells in the specified condition (refer to "culture conditions" table).

Cell Passage

1. Pre-warm the medium (Insect SFM01 Pro) in 27℃ water bath for 20 ~ 30 min.

2. Cells with viable cell density ≥ 2.5×10^6 cells/mL, cell viability ≥ 90%, and in the middle of logarithmic growth phase were selected for passage.

3. According to the seed cell density of 1.0×10^6 cells/mL, calculate the amount of total number of seed cells.

4. Seed cells at 1.0×10^6 cells/mL in the flask and add a certain volume of pre-warmed fresh medium.

5. Incubate cells in the specified environment condition (refer to "culture conditions" table).

6. Passage cells with fresh medium according to the above steps every 48 ± 3 hours.

7. If the viable cell density is less than 2.5×10^6 cells/mL or the viability is lower than 90% before passaging, the cells need to be centrifuged at 150 g ~ 300 g for 5 minutes. Carefully remove the spent media, then resuspend cells with preheated Insect SFM01 Pro medium, passage cells after sampling and counting.

Adaptation

Direct Adaptation

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

2. Cell passage until cell expression steadily.

3. When VCD reaches 2.5×10^6 cells/mL and > 90% viability（48 ± 3 hours）. At this point, the cells had been

successful adapted.

Sequential Adaptation

For cells growing in 5 ~ 10% serum or SFM media. Sequential adaptation should be performed.

1. Seed cells at 1.0×10^6 cells/mL in original cell culture media.

2. Sample and cell count every day until the VCD reaches 2.5×10^6 cells/mL.

3. Seed cell density at 1.0×10^6 cells/mL, subculture cells into stepwise increasing ratios of complete Insect SFM01 Pro medium to original medium with each subsequent passage (25:75, 50:50, 75:25, 90:10, 100:0).

4. When VCD reaches 2.5×10^6 cells/mL and ≥ 90% viability（48 ± 3 hours）. At this point, the cells had fully adapted to Insect SFM01 Pro media.

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% Insect SFM01 Pro + 10% DMSO, store at 4℃.

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

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

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

7. Transfer to liquid nitrogen tank for storage.