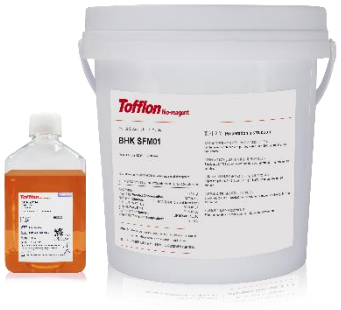
Version 2，2023

The User's Manual—BHK SFM01

Basic Information Introduction

Product Introduction

BHK SFM01 is serum-free media, without serum, protein, any animal-derived components and ect, formulated with high quality raw materials, high quality and stability, and no serum is required for the culture process, it suitable for high-density cell suspension expansion of BHK cell, supporting BHK rapid expansion, high-density culture and virus vaccine production under a completely serum-free protein-free system.



Application Scope

BHK SFM01 medium is suitable for suspension domestication and large-scale serum-free suspension culture of BHK cells. It can be used in the development and production process of rabies vaccine and other biological products, and can support the development to large-scale production, 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
BHK SFM01	LQ16, Liquid	2℃ ~ 8℃, Protect from light	2℃ ~ 8℃, Protect from light	12 months
BHK SFM01	DP16, 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 100% of the final volume.
2. Slowly add 24.724 g/L of powder medium with gentle stirring. Mix for 10 minutes.
3. Adjust the pH to 6.0 ~ 6.5 using 200 g/L NaOH solution. Mix for 20 minutes.
4. Slowly add 2.438 g/L $NaHCO_3$ to the solution with gentle stirring. Mix for 10 minutes.
5. Filter immediately the media with a 0.22 μm membrane filter.

Quality Index of Powder and Liquid Media

Product Index	BHK SFM01 (LQ16), Liquid	BHK SFM01 (DP16), Powder
Appearance	Yellow, clear liquid	Light yellow, light pink or similar color powder
pH	7.0 ~ 7.4	7.0 ~ 7.4 (pre-filter)
Osmolality (mOsmol/kg)	300 ~ 355	310 ~ 350
Solubility	--	Dissolve well according to the protocol for hydration of powder medium
Endotoxin (EU/mL)	< 10	< 10
Sterility	Negative	--
Bioburden	--	Aerobic bacteria: < 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	37℃
	Incubation CO_2 concentration	5%
	Incubation relative humidity	> 80% RH

Cell Thawing

1. Pre-warm the medium (BHK SFM01) in 37°C 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°C water bath. Gently agitate the vial within 1 minutes until the ice in the vial melting.

4. Pipet the contents from the vial gently into a centrifuge tube containing 30mL of pre-warmed medium (BHK SFM01).

5. Centrifuge 170 g ~ 190 g for 5 minutes. Discard the supernatant and re-suspend cells in 10 ~ 30mL fresh pre-warmed medium (BHK SFM01), 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 > 90%, incubate cells in the specified condition (refer to "culture conditions" table).

Cell Passage

1. Pre-warm the medium (BHK SFM01) in 37°C water bath for 20 ~ 30 min.

2. Cells with viable cell density ≥ 7×10^6 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 BHK SFM01 medium according to the above steps once every 48 ± 3 hours.

7. If the viable cell density is less than 7.0×10^6 cells/mL or the viability is lower than 90% before passaging, the cells need to be centrifuged at 170 g ~ 190 g for 5 minutes. Carefully remove the spent media, then resuspend cells with preheated BHK SFM01 medium, passage cells after sampling and counting.

Adaptation

Direct Adaptation

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

2. Cell passage until cell expression steadily.

3. When VCD reaches 7×10^6 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 7×10^6 cells/mL.

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

4. When VCD reaches 7×10^6 cells/mL and ≥ 90% viability（48 ± 3 hours）. At this point, the cells had fully adapted to BHK SFM01 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: 93% BHK SFM01 + 7% 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℃ decrease per minute).

7. Transfer to liquid nitrogen tank for storage.