BHK Cell Culture Products

Version 1.3

The BHK product line has been specifically developed to facilitate optimal growth of BHK-21 cells, as well as efficient production of foot-and-mouth virus and pseudorabies virus in suspension culture. This comprehensive range includes specialized formulations such as low-serum medium, serum-free medium, maintenance medium, and enhancer.

- BHK LSM 361 is a low-serum medium that effectively supports both BHK-21 cell growth and virus production. It is recommended to be used with 1% serum unless other usage instructions are provided.
- BHK SFM 363, 365, 834, 1385, 1396, and 1450 are serum-free media that support BHK-21 cell growth and virus production.
- BHK PM 835 is a maintenance medium used to offer efficient virus production.
- Enhancement of virus titer can be achieved by utilizing Feed V (10000X) enhancer.

Product	Catalog No.	Form	Package Sizes
BHK LSM 361	77020-361	Dry powder	2 L, 10 L, 100 L, Customized
BHK SFM 363	10302-363	Dry powder	2 L, 10 L, 100 L, Customized
BHK SFM 365	10302-365	Dry powder	2 L, 10 L, 100 L, Customized
BHK SFM 834	10302-834	Dry powder	2 L, 10 L, 100 L, Customized
BHK SFM 1385	10302-1385	Dry powder	2 L, 10 L, 100 L, Customized
BHK SFM 1396	10302-1396	Dry powder	2 L, 10 L, 100 L, Customized
BHK SFM 1450	10302-1450	Dry powder	2 L, 10 L, 100 L, Customized
BHK PM 835	10304-835.1	Dry powder	2 L, 10 L, 100 L, Customized
Feed V (10000X)	99153-23009	Liquid	100 mL, 500 mL

Component Information

Without phenol red, any animal-origin components.

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.

Storage and Stability

The dry powder medium should be stored at 2–8°C and protected from light. The product is stable for 24 months when unopened and stored properly. This product is very hygroscopic and should be kept in a dry environment away from moisture.
Feed V (10000X) should be stored at −20°C with stability of 12 months.

Prepare Liquid Medium from Dry Powder

BHK LSM 361 (77020-361), BHK SFM 363 (10302-363), BHK SFM 365 (10302-365), BHK SFM 834 (10302-834), BHK SFM 1385 (10302-1385), BHK SFM 1396 (10302-1396), BHK SFM 1450 (10302-1450)

1. Fill the mixing container with purified water (20–30°C) at 80% of the final volume.
2. Slowly add dry powder with gentle stirring. Mix for 30 minutes.
   Note: 25.94 g/L for 77020-361, 27.94 g/L for 10302-363, 27.94 g/L for 10302-365, 28.59 g/L for 10302-834, 28.66 g/L for 10302-1385, 27.64 g/L for 10302-1396, 28.54 g/L for 10302-1450.
3. 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.
4. Adjust the final volume with purified water. Mix for 10 minutes.
5. Filter the media using a membrane filter with 0.22 μm pore size immediately.

PM 835 (10304-835.1)

1. Fill the mixing container with purified water (20–

30°C) at 80% of the final volume.
2. Slowly add 19.22 g/L of dry powder medium with gentle stirring. Mix for 30 minutes.
3. Adjust the pH to 7.0–7.6 using 1 M–5 M NaOH or HCl. The pH may rise 0.1 to 0.2 after sterile filtration.
4. Adjust the final volume with purified water. Mix for 10 minutes.
5. Filter the media using a membrane filter with 0.22 μm pore size immediately.

Culture Conditions

Culture Type: Suspension
Culture Vessels: TPP tubes and shake flasks
Shaking Speed: For TPP tubes, it is recommended 200 rpm @50 mm orbital diameter.
For shake flasks, it is recommended 125–150 rpm @25 mm orbital diameter or 90–120 rpm @50mm orbital diameter.
Culture Temperature: 37°C
CO_2 Concentration: 5%
Relative Humidity: 80% RH

Recover Cells

BHK LSM 361, BHK SFM 363, BHK SFM 365, BHK SFM 834, BHK SFM 1385, BHK SFM 1396, BHK SFM 1450

1. Quickly thaw (<1 minutes) the cryopreserved tube in a 37°C water bath.
2. Transfer all cell fluids into a sterile centrifuge tube with 30 mL prewarmed BHK cell culture medium, centrifuge the cells at 1000 rpm (about 233 × *g*) for 5 minutes, discard the supernatant and resuspend the cells with required volume of prewarmed BHK cell culture medium to make sure a final density of 0.8–1.2 × 10^6 cells/mL.
3. Culture the cells referring to the “Culture Conditions”.
4. 24 ± 2 hours after recovery, centrifuge the cells at 1000 rpm (about 233 × *g*) for 5 minutes discard the supernatant and resuspend the cells with 30 mL of prewarmed BHK cell culture medium.
5. 48 ± 2 hours after recovery, passage cells referring to the “Passage Cells and Scale-up”.

Passage Cells and Scale-up

BHK LSM 361, BHK SFM 363, BHK SFM 365, BHK SFM 834, BHK SFM 1385, BHK SFM 1396, BHK SFM 1450

1. After 48 ± 2 hours of recovery, the cells in mid-log phase of growth can be passaged. Determine viable cell density (× 10^6 cells/mL) and viability (%).
2. When viable cell density is more than 3.0 × 10^6 cells/mL and viability is more than 95%, passage the cells at 0.4–0.6 × 10^6 cells/mL and culture referring to the “Culture Conditions”.
3. Before using for other purposes, cells should be at least 3 passages in BHK cell culture medium.

Adapt Cells to BHK cell culture medium

BHK LSM 361, BHK SFM 363, BHK SFM 365, BHK SFM 834, BHK SFM 1385, BHK SFM 1396, BHK SFM 1450

Direct Adaptation

1. Transfer cells in mid-log phase of growth from other medium into BHK cell culture medium directly at 0.4–0.6 × 10^6 cells/mL. Culture the cells referring to the “Culture Conditions”.
2. It is recommended to measure the cell density (× 10^6 cells/mL) and viability (%) daily during the first passage in BHK cell culture medium.
3. The viable cell density should reach about 4 × 10^6 cells/mL with >95% viability within 2 days of seeding culture. At this stage, culture is adapted to BHK cell culture medium.
4. The other experiments should be carried out after 3–5 continuous passages.

Note: BHK-21 cells from JSBio can be recovered in BHK SFM 1385 directly.

Sequential Adaptation

1. If the attitude of cells is poor during direct adaption, suggest using sequential adaptation.
2. Seed cells at 0.4–0.6 × 10^6 cells/mL. At each subsequent passage, dilute cells with stepwise decreasing the ratio of original medium to BHK cell culture medium (90:10, 75:25, 50:50, 25:75, 0:100).
3. If viable cell density is around 3 × 10^6 cells/mL and viability is >95%, next step can be carried out. 2–3 passages at each step are needed to achieve consistent growth.
4. If the viable cell density in 100% BHK cell culture medium reaches 4 × 10^6 cells/mL with >95% viability after 2 days of seeding culture, cells are adapted to BHK cell culture medium.
5. Carry out other experiments with BHK-21 cells after a minimum of 3–5 passages of successful adaptation.

Cryopreservation

BHK LSM 361, BHK SFM 363, BHK SFM 365, BHK SFM 834, BHK SFM 1385, BHK SFM 1396, BHK SFM 1450

It is recommended to use cells in mid-log phase of growth with >95% viability for cryopreservation.

1. Prepare cryopreservation medium with 60% BHK cell culture medium + 30% FBS + 10% DMSO and store at 4°C until use.
   Note: Add DMSO finally to avoid turbidity.
2. Determine the cell density and viability, and calculate the required volume of cell cultures to give a final cryopreservation density of 30.0 × 10^6 cells/mL.
3. Centrifuge the cells at 1000 rpm (about 233 × *g*) for 5 minutes and discard the supernatant, then resuspend the pellet in the pre-determined

volume of cryopreservation medium.
4. Aliquot the suspension into 1 mL per cryovial, then transfer them into a pre-cooled freezing container immediately and place at −80°C for 24 hours.
5. Transfer frozen cells to liquid nitrogen for long-term storage.

Expression of Foot-and-mouth Virus

BHK LSM 361, BHK SFM 363, BHK SFM 365, BHK SFM 834, BHK SFM 1385, BHK SFM 1396, BHK SFM 1450, Feed V

The following method is used for early production only. Optimization of infection and feed processes is encouraged to obtain higher virus titer.

1. The viable cell density before infection should be reached 4–6 × 10^6 cells/mL with >95% viability.
2. Transfer fluids into a sterile centrifuge container and centrifuge at 1000 rpm (about 233 × *g*) for 5 minutes, discard the supernatant. Resuspend the cells with prewarmed BHK cell culture medium without serum and adjust the pH to 7.4 using 1M sodium bicarbonate solution.
   Note: If infect BHK-21 cells in a bioreactor, sediment cells overnight at 2–8°C, drain the supernatant, then add BHK cell culture medium without serum, adjust the pH to 7.4 using 1M sodium bicarbonate solution.
3. Infect BHK-21 cells with foot-and-mouth virus.
4. Supplement Feed V (10000X) to 1X, culture the cells referring to the “Culture Conditions”.
5. 12–16 hours post-infection, harvest BVDV according to cytopathic effect.

Related Products

Product	Catalog No.
Glucose Solution (300 g/L)	99024-16023
L-Glutamine (200 mM)	99025-17029