BHK Series Products

Version 2.0

The BHK Series Products are specifically designed for **BHK-21 cells**, supporting high-density suspension culture and enabling efficient virus production for pathogens such as Foot-and-Mouth Disease (FMD) and Pseudorabies (PRV) and Newcastle disease virus (NDV). This series includes Serum-Free Media, Expression Maintenance Medium, and a Virus Production Enhancer.

- **BHK SFM 1522:** A serum-free, animal component-free (ACF) medium formulated to support robust **cell growth**.
- **BHK SFM 2158:** A serum-free, animal component-free (ACF) medium designed to support both **cell growth** and **viral expression**.
- **Feed F (10,000X):** A Virus Production Enhancer designed to effectively boost viral yields.
- **Virus Production Enhancer (100X):** A Virus Production Enhancer designed to effectively boost viral yields.

Product Name	Catalog No.	Form	Product Series	Product Usage
BHK SFM 1522	10302-1522	Dry powder	Serum-Free Media	Cell Growth
BHK SFM 2158	10302-2158	Dry powder	Serum-Free Media	Cell Growth & Virus Expression
Feed F	99154-23010	Liquid	Supplement	Virus Production Enhancer
Virus Production Enhancer	99222-26019	Liquid	Supplement	Virus Production Enhancer

Safety Warning

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

Intended Use

For research and further manufacturing use only.

Storage and Stability

Store the dry powder medium at 2–8℃, protected from light and moisture. The unopened product has a shelf life of 24 months under recommended storage conditions. As the powder is highly hygroscopic, keep the container tightly sealed in a dry environment.

Feed F (10,000X) must be stored at −20℃. Virus Production Enhancer should be stored at 2–8℃.

Important: Do not use either product if particulate matter or turbidity is observed.

Prepare Liquid Medium from Dry Powder

BHK SFM 1522(10302-1522)

- Fill the mixing container with purified water (20–30℃) at 80%–90% of the final volume.
- Slowly add 28.64 g/L of dry powder medium with gentle stirring. Mix for 30 minutes.
- Adjust the pH to 6.8–7.0 using 1M–5M NaOH or HCl. The pH may rise 0.1 to 0.2 after sterile filtration.
- Adjust the final volume with purified water. Mix for 10 minutes.
- Filter the media using a membrane filter with 0.22 μm pore size immediately.

BHK SFM 2158(10302-2158)

- Fill the mixing container to about 80% of the final volume with purified water (room temperature), start stirring.

- Slowly add 29.13 g/L of dry powder medium. Mix until there is no dry powder on the surface of the solution and stir for 30 minutes.
- Slowly add 2.40 g/L of sodium bicarbonate (CAS:144-55-8). Mix for 10 minutes.
- Adjust the pH to 7.0–7.4 with 1 M–5 M HCl or NaOH solution. Mix for 10 minutes. The pH may rise 0.1 to 0.2 after sterile filtration.
- Adjust the final volume with purified water (density: 1.0 0 g/mL). Mix for 10 minutes.
- Filter the medium immediately using a membrane filter with 0.22 μm pore size.

Culture Conditions

- **Culture Type:** Suspension
- **Culture Vessels:** Shake flasks
- **Shaking Speed:** 125–150 rpm@25 mm orbital diameter or 90–120 rpm@50 mm orbital diameter.
- **Culture Temperature:** 37℃
- **CO_2 Concentration:** 5%
- **Relative Humidity:** 80% RH

Recover Cells

- **Thawing:** Rapidly thaw the cryopreserved vial in a 37℃ water bath for 1 to 2 minutes.
- **Centrifugation and Resuspension:**
  a. Aseptically transfer the entire cell suspension into a sterile centrifuge tube containing 30 mL of pre-warmed BHK-21 Series Medium.
  b. Centrifuge at 1000 rpm (approximately 233 × g) for 5 minutes.
  c. Carefully discard the supernatant.
  d. Resuspend the cell pellet in an appropriate volume of pre-warmed BHK-21 Series Medium. to achieve a final seeding density of (0.8–1.2) × 10^6 cells/mL.
  e. Transfer the cell suspension into a new shake flask.
  f. After 24 ± 2 h of recovery, harvest the cells by centrifugation at 1000 rpm (approx. 233 × g) for 5 minutes. Discard the supernatant, resuspend the cell pellet in 20 mL of pre-warmed medium, and transfer to a sterile shake flask.
- **Initial Culture:** Incubate the cells under the conditions specified in the "**Culture Conditions**" section.
- **Routine Passaging:** Beginning with the first full passage after recovery, subculture the cells every 48 ± 2 h by following the steps outlined in "**Cell Passage and Scale-up**".

Cell Passage and Scale-up

- **Pre-Passage Assessment:** At 48 ± 2 h post-recovery or post-previous passage, determine the cell density and viability.
- **Passaging Criteria and Execution:**
  a. When the viable cell density reaches at least 3.0 × 10^6 cells/mL with viability exceeding 90%, subculture the cells by seeding them at a density of (0.6–0.8) × 10^6 cells/mL in BHK-21 Series Medium. If the viable cell density is < 3.0 × 10^6 cells/mL or the cell viability is < 90%, perform centrifugal subculture by centrifuging the cell suspension at 233 × g (approximately 1000 rpm) for 5 minutes, discarding the supernatant, and subculturing at a seeding density of (0.6–0.8) × 10^6 cells/mL.
  b. Culture the newly seeded cells according to the "**Culture Conditions**".
- **Adaptation Requirement:** Prior to use in other applications, cells must undergo a minimum of 3 consecutive, successful passages in BHK-21 Series Medium to ensure proper adaptation.

Adaptation to BHK-21 Series Medium

Direct Adaptation

- **Initial Seeding:** Directly transfer cells from their current medium into BHK-21 Series Medium at a seeding density of (0.6–0.8) × 10^6 cells/mL. Culture the cells according to the specifications in the “Culture Conditions” section.
- **Monitoring:** Following the initial transfer, it is recommended to monitor the viable cell density (× 10^6 cells/mL) and viability (%) daily.
- **Success Criterion:** Adaptation is considered successful when, after several consecutive passages, the cell growth profile (including doubling time and maximum density) aligns consistently with or exceeds the historical data from the client's existing process.
- **Passaging and Other Experiments:**
  a. Perform routine passaging by following the procedures outlined in “**Cell Passage and Scale-up**”.
  b. Cells should undergo a minimum of 3 to 5 consecutive, stable passages in BHK-21 Series Medium before being used for other experiments.

Sequential Adaptation

- **Initial Seeding:** Begin by seeding BHK-21 cells at a viable cell density of (0.6–0.8) × 10^6 cells/mL in a mixture of the original medium and BHK-21 Series Medium.
- **Gradual Media Transition:** During each subsequent passage, gradually reduce the proportion of the original medium while increasing that of BHK-21 Series Medium, transitioning sequentially through the following ratios: 90:10 (Passage 1) → 75:25 (Passage 2) → 50:50 (Passage 3) → 25:75 (Passage 4) → 0:100 (Passage 5, representing 100% BHK-21 Series Medium).
- **Process Alignment & Verification:**
  a. Once the cell growth profile demonstrates alignment with the trend of the client's established process, conduct an additional 2 to 3 passages under the same conditions.
  b. Proceed to the next stage only if consistent and reproducible growth is maintained throughout this verification period.
- **Final Adaptation in 100% Medium:** Continue passaging the cells in 100% BHK-21 Series Medium. Successful adaptation is confirmed when the growth profile consistently matches or exceeds the client’s process trend, which typically requires a minimum of 3 to 5 successful passages in the 100% medium. Upon meeting this criterion, the cells are ready for use in other experiments.
  Note：The section "**Adaptation to BHK-21 Series Medium** " applies only when transferring the customer's cells into BHK-21 Series Medium.

Cryopreservation

- **Recommended Cell State：**
  Use cells in mid-logarithmic growth phase (approximately 48 ± 2 h post seeding) with viability > 95%.
- **Procedure：**
  Prepare cryopreservation medium (60% BHK-21 Series Medium + 30%NBCS + 10% DMSO) and store at 2–8℃ until use.
  a. Determine cell density and viability, then calculate the volume needed to obtain a final concentration of 3.0 × 10^7 cells/mL.
  b. Harvest cells by centrifugation (1,000 rpm ≈ 233 × g, 5 min), discard the supernatant, and resuspend the pellet in the pre-calculated volume of chilled cryopreservation medium.
  c. Aliquot 1 mL per cryovial, place vials into a pre-cooled freezing container, and transfer to a -80℃ freezer for ≥ 24 h.
  d. For long-term storage, transfer frozen vials to the vapor phase of liquid nitrogen.

Virus Production (FMDV)

- **Scope**
  This section outlines the baseline method for initial virus production. Process optimization (e.g., seeding density, infection timing, harvest time) is recommended to achieve higher titer.
- **Experiment Design**
  To avoid overloading the downstream purification equipment, we are offering two options for infecting the cells. Please select the scheme that matches your facility's processing capacity.
  Scheme1(Low-Density Infection):

Growth Medium	Maintenance Medium	Infection Conditions	Culture Conditions
BHK SFM 2158	BHK SFM 2158 + 1.15X Virus Production Enhancer	Infection Density: (5–8) × 10^6 cells/mL Inoculum Volume Ratio: 1/1000–5/1000 Infection pH: 7.6–8.0	Refer to "**Culture Conditions**"

  Scheme2(High-Density Infection):

Growth Medium	Maintenance Medium	Infection Conditions	Culture Conditions
BHK SFM 1522	BHK SFM 2158+1.15X Virus Production Enhancer	Infection Density: ＞8 × 10^6 cells/mL Inoculum Volume Ratio: 1/1000–5/1000 Infection pH: 7.6–8.0	Refer to "**Culture Conditions**"

- **Experimental Methods**
  a. Count the cells cultured for 48 h in the shaker flask using a cell counter to measure cell density (× 10^6 cells/mL) and viability (%).
  b. Calculate the required cell volume based on the infection density specified in the experimental design. Centrifuge at 1000 rpm for 5 min to remove the supernatant, then resuspend the cells in the corresponding maintenance medium.
  c. Adjust the pH of the cell culture medium to the pH set by the infection process (generally set between 7.6–8.0) using 18% (w/v) Na_2CO_3. Add the seed virus at a volume ratio of 1/1000–5/1000 (or refer to the current process) and mix well.
  d. Add Feed F (10,000X concentrate) to a final working concentration of 1X.
  e. Culture the cells according to " **Culture Conditions**".
  f. After infection, monitor the pH every 2 h. When pH < 7.6, add 18% (w/v) dropwise along the vessel wall, mix gently, and maintain the pH at 7.8 ± 0.1. The volume of base added must be recorded in real-time.
  g. Observe Cytopathic Effect (CPE) during the process. Harvest the virus according to the client's existing process (generally harvest when CPE > 90%).

Virus Production (NDV)

- **Scope**
  This section outlines the baseline method for initial virus production. Process optimization (e.g., seeding density, infection timing, harvest time) is recommended to achieve higher titer.
- **Experiment Design**
  Scheme1(Low-Density Infection):

Growth Medium	Maintenance Medium	Infection Conditions	Culture Conditions

BHK SFM 2158	BHK SFM 2158 BHK SFM 2159	Infection Density: (4–6) × 10^6 cells/mL Inoculum Volume Ratio: 1/1000–5/1000 Temperature: 33–35℃	Refer to "**Culture Conditions**"

Scheme2(High-Density Infection):

Growth Medium	Maintenance Medium	Infection Conditions	Culture Conditions
BHK SFM 1522 BHK SFM 2159	BHK SFM 2158	Infection Density: 6–10 × 10^6 cells/mL Inoculum Volume Ratio: 1/1000–5/1000 Temperature: 33–35℃	Refer to "**Culture Conditions**"

- **Experimental Methods**

  a. Count the cells cultured for 48 h in the shaker flask using a cell counter to measure cell density (× 10^6 cells/mL) and viability (%).

  b. Calculate the required cell volume based on the infection density specified in the experimental design. Centrifuge at 1000 rpm for 5 min to remove the supernatant, then resuspend the cells in the corresponding maintenance medium.

  c. Add the seed virus at a volume ratio of 1/1000–5/1000 (or refer to the current process) and mix well.

  d. Add 5–10mg/L TPCK and adjust the temperature to 33–35℃.

  e. Begin sampling and preserving samples every 24 hours starting from 48 hours post-infection, for HA assay.

Virus Production (PRV)

- **Scope**

  This section outlines the baseline method for initial virus production. Process optimization (e.g., seeding density, infection timing, harvest time) is recommended to achieve higher titer.

- **Experiment Design**

  Scheme1(Low-Density Infection):

Growth Medium	Maintenance Medium	Infection Conditions	Culture Conditions
BHK SFM 2158	BHK SFM 2158 BHK SFM 2159	Infection Density: (4–6) × 10^6 cells/mL Inoculum Volume Ratio: 1/1000–5/1000	Refer to "**Culture Conditions**"

Scheme2(High-Density Infection):

Growth Medium	Maintenance Medium	Infection Conditions	Culture Conditions
BHK SFM 1522 BHK SFM 2159	BHK SFM 2158	Infection Density: 6–10 × 10^6 cells/mL Inoculum Volume Ratio: 1/1000–5/1000	Refer to "**Culture Conditions**"

- **Experimental Methods**

  a. Count the cells cultured for 48 h in the shaker flask using a cell counter to measure cell density (× 10^6 cells/mL) and viability (%).

  b. Calculate the required cell volume based on the infection density specified in the experimental design. Centrifuge at 1000 rpm for 5 min to remove the supernatant, then resuspend the cells in the corresponding maintenance medium.

  c. Add the seed virus at a volume ratio of 1/1000–5/1000 (or refer to the current process) and mix well.

  d. Post-inoculation, cell density, viability, and CPE are monitored starting at 24 h. From 24 h to 42 h post-infection,

samples are collected every 6 h for viral titer ($TCID_{50}$) determination. The optimal harvest time is determined based on the kinetic changes in viral titers over this period.