Celhappy® BHK LSM 361

Version 2.0

Celhappy® BHK LSM 361 medium is a low serum medium specially developed for the high-density suspension culture of baby Hamster Syrian Kidney (BHK-21) cells and the production of animal vaccines. It does not contain protein, peptides, and animal-derived components. It suitable for large-scale industrial production of BHK-21 cells.

Product	Catalog No.	Form	Package Sizes
BHK LSM 361	77020-361	Dry powder	5 L, 10 L, 50 L, 100 L, 500 L, Customized

Component Information

With sodium bicarbonate, 6.2 g/L glucose, 6.0 mM glutamine; Without phenol red.

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.

Prepare Liquid Medium from Dry Powder

1. Fill the mixing container with purified water (20–30℃) at 80%–90% of the final volume.
2. Slowly add 25.94 g/L of dry powder medium with gentle stirring. Mix for 30 minutes.
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.

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

1. Quickly thaw the cryopreserved tube into a 37°C water bath, rapid thaw (1–2 minute) the frozen cells.
2. Transfer all cell fluid into a sterile centrifuge tube with 30 mL prewarmed BHK LSM 361+1% NBCS (Newborn Calf Serum) medium, centrifuge the cells at 1000 rpm (about 233 g) for 5 minutes, aspirate and discard the supernatant, transfer them into a new TPP tube at 0.8–1.2 × 10^6 cells/mL.
3. Culture the cells referring to the “Culture Conditions”.

Passage Cells and Scale-up

1. After 48 ± 6 hours of recovery, the cells were at the middle of the logarithmic growth phase could be passage.
2. Determine viable cell density (× 10^6 cells/mL) and viability (%).
3. When viable cell density more than 4.0 × 10^6 cells/mL and viability more than 90%. Passage the cells at 0.5–0.6 × 10^6 cells/mL and culture referring to the “Culture Conditions”.
4. Before using for other purposes, cells should be at least 3 passages in BHK LSM 361 medium.

Cryopreservation

It is recommended to use cells in mid-logarithmic growth phase with >90% viability for cryopreservation.

1. Use a cryopreserved medium contained 60% BHK LSM 361 media + 30% NBCS + 10% DMSO.
2. According to the cryopreserved volume is 1.0 mL/ tube, the cryopreserved density is 3.0 × 10^7 cells/mL.

3. Centrifuge the cells at 1000 rpm (about 233 g) for 5 minutes, aspirate and discard the supernatant. Adding the required volume of cell cryopreserved medium to prepared cell cryopreserved suspension.
4. Cell cryopreserved suspension was divided into each cryopreserved tube and immediately transferred to the pre-cooled freezing container, after the programmed cooling, transfer them to the liquid nitrogen tank for long-term storage.

Infect the FMD Virus in Suspension BHK-21 Cells

After 3–5 continuous passages in BHK LSM 361 medium, the infection experiment could be carried out. And in the infection experiment, 1% serum needs to be added in BHK21 LSM 361 medium for cell growth, and no serum is required in the viral expression stage. That following is the detailed steps:

1. On the day of passage, determine viable cell density (x 10^6 cells/mL) and viability (%) using cell counter equipment. Calculate the required volume of BHK LSM 361 medium, serum and the cell fluid, according to the seeding density of 0.4–0.6 × 10^6 cells/mL.
2. Mix the required cell fluid, prewarmed BHK LSM 361 medium and serum according to the calculation results, conduct batch culture. Two parallel conditions are recommended.
3. The viable cell density can reach about 4–5 x 10^6 cells/mL (Shaker Flasks) and about 6–8 x 10^6 cells/mL (Bioreactor) on the 48 hours of batch culture (Shaker Flasks), at which time for virus infection.
4. Centrifuge all the cells at 150–300 g (about 800–1150 rpm) for 5 minutes, aspirate and discard the supernatant, then resuspend cells with 100% fresh prewarmed BHK LSM 361 medium according to the cell density of 5–6 x 10^6 cells/mL.
5. The recommended inoculation ratio of the virus is the 1–2‰‰ of working volume (virus volume: working volume = 1–2:1000).
6. The viruses can be harvested if the cytopathic effect (CPE) >90%. Generally, viruses can be harvested in 12–16 hours for shake flasks culture, 12–14 hours for bioreactor culture.

Related Products

Product	Catalog No.
BHK SFM 1385	10302-1385
Feed V (10000X)	99153-23009