MetaCell® BHK-200 Chemically Defined Medium

User Manual

Product Description

MetaCell® BHK-200 is a chemically defined, serum-free medium specifically designed for the high-density suspension culture of BHK-21 cells, suitable for the research and development and large-scale production of vaccines based on BHK-21 cells. This medium enables BHK-21 cells to rapidly acclimate from serum-containing to serum-free conditions while maintaining high cell density and high cell viability.

MetaCell® BHK-200 contains 6mM L-glutamine.

This product is intended for research or further manufacturing but not for human or therapeutic use.

Product Name	Cat No.	Form	Size	Storage	Shelf Life	Application
MetaCell® BHK-200	P3102-X100	Powder	100L	2-8°C, protected from light	12 months	high-density suspension culture of BHK-21 cells
	P3102-X500		500L			

Cell Culture Conditions

Basal medium: MetaCell® BHK-200
Application: Suspension cell culture
Cell line: BHK-21, BHK
Recommended set-up for initial trials:

Vessel volume	125mL	250mL	500mL	1L
Medium volume	30-35	60-70	120-140	240-380
Shaker speed	155±5 rpm (amplitude 19mm) 150± 5 rpm (amplitude 25mm) 130± 5 rpm (amplitude 50mm)			
Types of flasks	PETG or PC, breathable, without baffles			
Culture environment	37 ± 0.5 ℃, 5% CO_2, humidity ≥80%, Ensure proper gas exchange and minimize light exposure during cultivation			

General instructions

Powdered media are hygroscopic and should be protected from moisture. The entire contents of each package should be used immediately after opening. Preparing a concentrated solution of medium is not recommended as precipitates may form.

Media preparation instruction by weight (1kg of final net weight of liquid medium)

1. Add 930-950g of ultrapure water or water for injection (temperature at 20-30 °C) into a clean container.
2. Weigh out 21.563g-21.649 g of powder medium, slowly add it to the container and stir until no lumps are present. The labeled amount of the medium is 21.606 g/L.
3. Add 6.0 mL of 5 mol/L sodium hydroxide solution and stir for 5 minutes until the solution has become clear.
4. Add 2.096-2.104g of sodium bicarbonate to the solution, stir for 20-25 minutes until the sodium bicarbonate is completely dissolved. The final concentration of sodium bicarbonate should be 2.100g/L.
5. Adjust the pH to the desired range (recommended PH 6.95-7.05) using 5mol/L hydrochloric acid solution.
6. Add water to a net weight of 998-1002g and stir for 5-10 minutes. If there is a significant change in pH, continue adjusting the pH to the final range of 6.95-7.05 using 5mol/L sodium hydroxide solution or 5mol/L hydrochloric acid solution.
7. Use a 0.22 μm sterilization-grade filter membrane for sterilization and filtration into a suitable container, and store it in a sealed and light-proof manner at 2-8 °C.

Cell Recovery

1. For a cryopreserved cell density of 15.0-18.0×10^6 cells/mL with a recovery volume of 20 mL as an example, take 29 mL of MetaCell® BHK-200 and place it in a 125 mL shake flask, pre-warming it at 37°C for 20-30 minutes.
2. Remove a vial of cryopreserved cells from the liquid nitrogen storage tank and place it in a 37°C water bath for rapid thawing (less than 1 minute). Take out the vial when the ice block inside is almost completely melted.
3. Add 9 mL of pre-warmed MetaCell® BHK-200 into a 15 mL centrifuge tube, then transfer the thawed cells into this tube and gently mix by inversion. After mixing, sample the suspension to measure the cell density and viability.
4. Centrifuge an appropriate amount of the cell suspension at 1000 rpm for 4 minutes. Discard the supernatant and resuspend the pelleted cells in pre-warmed MetaCell® BHK-200. It is recommended to achieve a final seeding density of 0.5-0.7×10^6 cells/mL in a total culture volume of 20 mL within the 125 mL shake flask.
5. Place the shake flask on a cell culture shaker with recommended parameters set at 37°C, 5% CO_2, and 130 ± 5 rpm (with an amplitude of 50 mm). The target cell density on D2 should be 3.0-5.0×10^6 cells/mL.
6. When the cell viability is ≥95%, proceed with subculture. It is recommended to perform at least three passages before conducting subsequent experiments.

Cell Passaging

1. Pre-heat MetaCell® BHK-200 at 37°C for 20-30 minutes.
2. When the cell density reaches 4.0-6.0×10^6 cells/mL and the cell viability is ≥95%, passaging can be performed.
3. The recommended seeding density for passaging is 0.5-0.7×10^6 cells/mL.
4. Transfer the required amount of seed solution to the shake flask, add an appropriate amount of pre-heated medium, set the parameters of the shaker according to the culture conditions, and passage the cells every 2 days using fresh medium following the above steps.
5. Cells should be passaged at least three times after thawing and recovery before subsequent experiments.

Cell Cryopreservation

1. Prepare a sufficient number of cells in the early logarithmic growth phase with a cell viability ≥95% for cryopreservation.
2. The final cell concentration for cryopreservation should be adjusted to 15.0-18.0×10^6 cells/mL.
3. Pre-cool the cryopreservation solution (90% MetaCell® BHK-200 + 10% DMSO) at 2-8°C for at least 30 minutes.
4. Take an appropriate amount of cell suspension, centrifuge at 1000rpm for 4 minutes, discard the supernatant, and resuspend the cells in the pre-cooled cryopreservation solution.
5. Divide the cell suspension into cryotubes according to the cryopreservation specifications.
6. Gradually cool the cells to -80°C for freezing (cooling rate of 1°C/min) using a controlled-rate freezer or manual control method.
7. After 24 hours, transfer the frozen cells to the vapor phase of a liquid nitrogen tank (storage temperature range: -200°C to -125°C) for storage.

Cell Adaptation

In most cases, serum-free cultured BHK-21 cells can be directly adapted to MetaCell® BHK-200. If direct replacement of the medium (direct adaptation) fails, it is recommended to use gradient replacement (indirect adaptation) to adapt BHK-21 cells to MetaCell® BHK-200.

Note: BHK-21 cells used for adaptation need to be in the early logarithmic growth phase, with a cell viability≥95%.

• Direct Adaptation Method

For BHK-21 cells that can be directly adapted, when the cell viability is ≥95% and in the early logarithmic growth phase, try directly transferring from the current serum-free medium to MetaCell® BHK-200.

1. Inoculate the cells into fresh MetaCell® BHK-200 at a seeding density of 0.5-0.7×10^6 cells/mL (refer to the cell passaging steps).
2. After 2 days of culture, check the cell density and viability. At this time, the cell viability should be ≥90%. If the viability is lower, use the indirect adaptation method described below.
3. Continue to passage the cells for 3-5 times. When the cell density reaches 4.0-6.0 x 10^6 cells/mL, and cell viability is ≥95%, the cells can be considered fully adapted.

Note: After 3-5 passages, if the cells still cannot resume normal growth, please switch to the indirect adaptation method described below.

• Indirect Adaptation Method

1. Mix the original medium and MetaCell® BHK-200 at a volume ratio of 75:25, and the seeding cell density should be 0.5-0.7 ×10^6 cells/mL.
2. Cells should be passaged when the cell density reaches 4.0-6.0 ×10^6 cells/mL after culturing for 2 days.
   (1) If the cells grow well and the viability is ≥90%, adjust the ratio of MetaCell® BHK-200 to the original medium to 50:50 during passaging.

(2) If the cells grow slowly, cells should be collected by centrifugation at 1000rpm for 4 minutes. Resuspended the cells in fresh mixed medium. The medium mix at this point still consists of MetaCell® BHK-200 and the original medium at a ratio of 25:75.

3. Repeat step 2 and gradually increase the ratio of MetaCell® BHK-200 (50:50, 75:25) until 100% MetaCell® BHK-200 is used for cell culture.
4. Continue culturing the cells in 100% MetaCell® BHK-200 for 3-5 passages. When the cell density reaches 4.0-6.0×10^6 cells/mL within 3-4 days and the cell viability is ≥95%, the adaptation is considered complete.
5. Continue the passaging for at least 3 times. If the cell growth remains stable, subsequent experiments can be conducted.