MetaCell® CHO-500 Chemically Defined Medium

User Manual

Product Description

MetaCell® CHO-500 is a chemically defined cell culture medium designed to maximize recombinant protein expression in Chinese Hamster Ovary (CHO) cell culture. Free from hydrolysates, proteins, growth factors and any animal-derived components, this medium is tailored to accommodate diverse CHO cell lines in high-density fed-batch processes, ensuring robust and high-yield protein expression. Synergistic use with MetaCell® Feed-500A High-Glucose and MetaCell®Feed-500B is recommended to maximize the cell culture performance.

MetaCell®CHO-500 is intended for research or further manufacturing but not for human or therapeutic use.

MetaCell®CHO-500 contains no L-glutamine.

Product Name	Product	Form	Size	Storage	Shelf Life	Application
MetaCell® CHO-500	P1010-X010	Powder	10L	2-8°C, protected from light	12 months	Fed-batch cell culture with CHOK1, DG44, CHO-S cells
	P1010-X100		100L			
	P1010-X500		500L			

Cell Culture Conditions

Basal medium: MetaCell®CHO-500
Application: Suspension cell culture
Cell line: CHO-GS, CHO-K1, CHO-DG44, CHO-S
Recommended set-up for initial trials:

Vessel volume	125mL	250mL	500mL	1L
Medium volume	25-35	60-80	120-160	240-300
Shaker speed	140±5 rpm (amplitude 19mm) 135± 5 rpm (amplitude 25mm) 105± 5 rpm (amplitude 50mm)			
Types of flasks	PETG or PC, breathable, without baffles			
Culture environment	36.5 ± 0.5 ℃, 5-8% 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.

MetaCell® CHO-500 contains no L-glutamine or its derivates. Please add L-glutamine or its derivates according to your needs. Please find recommended products at the end of this document.

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

1. Add 900-920g of ultrapure water or water for injection (temperature at 20-30 °C) into a clean container.
2. Weigh out 20.996g-21.081 g of powder medium, slowly add it to the container and stir until no lumps are present. The labeled amount of the medium is 21.039 g/L.
3. Add 8.0 mL of 5 mol/L sodium hydroxide solution and stir for 5 minutes until the solution has become clear.
4. Add 1.796-1.804g 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 1.800g/L.
5. Adjust the pH to the desired range (recommended PH 6.95-7.3) 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 6.95-7.3 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. Cells transported on dry ice should be placed in a liquid nitrogen environment for 3-7 days before cell recovery.
2. Preheat the MetaCell® CHO-500 medium at 37 ℃.
3. Take a vial of frozen cells from the liquid nitrogen tank and thaw in a 37°C water bath (1-2 minute).
4. Transfer the cells to a 15mL centrifuge tube containing 9 mL of pre-heated MetaCell® CHO-500.
5. Centrifuge at 1000rpm for 4 minutes, discard the supernatant, resuspend the cells in pre-heated MetaCell® CHO-500, and transfer them to a 125mL shake flask. Add MetaCell® CHO-500 to adjust the final volume to 30mL.
6. After 3-5 days of cultivation, the viable cell density (VCD) should reach≥ 3.0 x 106 cells/mL and the viability≥ 90%.
7. We recommend to passage the culture for at least three passages before starting subsequent experiments.

Cell Passaging

1. Pre-heat MetaCell® CHO-500 at 37°C for 20-30 minutes.
2. When the cell density reaches 3.0-5.0×10^6 cells/mL and the cell viability is ≥95%, passaging can be performed.

   Note: Different CHO cell lines may have different ranges of logarithmic growth phases, and the passaging time needs to be determined according to the actual situation to ensure that passaging culture is carried out in the early logarithmic growth phase.
3. The recommended seeding density for passaging is 0.4-0.6×10^6 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 3-4 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 1.0×10^7 cells/mL.
3. Pre-cool the cryopreservation solution (90% MetaCell® CHO-500 + 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 CHO cells can be directly adapted to MetaCell® CHO-500. If direct replacement of the medium (direct adaptation) fails, it is recommended to use gradient replacement (indirect adaptation) to adapt CHO cells to MetaCell® CHO-500.

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

• Direct Adaptation Method

For CHO 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® CHO-500.

1. Inoculate the cells into fresh MetaCell® CHO-500 at a seeding density of $0.4\text{-}0.6\times10^6$ cells/mL (refer to the cell passaging steps).
2. Passage the cells every 3- 4 days for at least 3-5 passages
3. After 3 -4 days of culture, check the cell density and viability. At this time, the cell viability should be ≥95%. If the viability is lower, use the indirect adaptation method described below.
4. Continue to passage the cells for 3-4 times. When the cell density reaches 3.0-5.0 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® CHO-500 at a volume ratio of 75:25, and the seeding cell density should be 0.4-0.6 $\times 10^6$ cells/mL.
2. Cells should be passaged when the cell density reaches 3.0-5.0 $\times 10^6$ cells/mL after culturing for 3 - 4 days.
   (1) If the cells grow well and the viability is >90%, adjust the ratio of MetaCell® CHO-500 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® CHO-500 and the original medium at a ratio of 25:75.
3. Repeat step 2 and gradually increase the ratio of MetaCell® CHO-500(50:50, 75:25) until 100% MetaCell® CHO-500 is used for cell culture.
4. Continue culturing the cells in 100% MetaCell® CHO-500 for 3-5 passages. When the cell density reaches 3.0-5.0×10^6 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.

Related Products

Products	Product Type	Form	Cat No.	Size
MetaCell® CHO-500	Basal Medium	Liquid	L1010-1000	1000mL
		Powder	P1010-X010	10L
			P1010-X100	100L
			P1010-X500	500L
MetaCell® Feed-500B	Feed B	Liquid	L1012-0100	100mL
		Powder	P1012-X001	1L
			P1012-X010	10L
MetaCell® Feed-500A High Glucose	Feed A	Liquid	L1017-0100	100mL
			L1017-0500	500mL
		Powder	P1017-X001	1L
			P1017-X010	10L
			P1017-X050	50L