MetaCell® CHO-320 Chemically Defined Medium

Product Description

MetaCell® CHO-320 is a serum-free, chemically defined cell culture medium designed for high-density transient transfection of CHO cells. It supports both small-scale and large-scale transient chemical transfections of CHO cells and is compatible with a variety of commercial cationic transfection reagents. It is recommended to use it in conjunction with MetaCell® Titer Enhancer and MetaCell® CHO TransFeed. For transfection reagents, PolyPlus MetaCell® or PEI 40K are recommended.

MetaCell® CHO-320 contains 6mM glutamine derivative.

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® CHO-320	P1015-X010	Powder	10L	2-8°C, protected from light	12 months	Efficient transient transfection of CHO cells
	P1015-X100		100L			

Cell Culture Conditions

Medium: : MetaCell® CHO-320

Application: Suspension cell culture

Cell line: ExpiCHO-S 、CHO-K1

Recommended parameters for trials:

Shake flask volume	125mL	250mL	500mL	1L	3L	5L
Medium volume	30-35mL	60-70mL	120-140mL	240-280mL	600-1000mL	1500-2000mL
Shaker speed	125±5 rpm (amplitude19mm) 120± 5 rpm (amplitude25mm) 95±5 rpm (amplitude50mm)				105±5 rpm 95±5 rpm 80±5 rpm	
Types of flasks	PETG or PC, breathable, without baffles					
Culture environment	37 ± 0.5 ℃, 8% CO_2, humidity ≥80%, Ensure proper gas exchange and minimize light exposure during cultivation					

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

1. Add 880-900g of ultrapure water or injectable water (water temperature 20-30°C) into a clean container.
2. Weigh out 21.364-22.227g of the MetaCell® CHO-320 and slowly pour it into the container (The final concentration of the medium should be 21.792 g/L).
3. Add 2.096-2.104g of sodium bicarbonate (labeled amount: 2.100g/L), and stir until completely dissolved.
4. Adjust the pH to the desired range using 5mol/L sodium hydroxide solution or 5mol/L hydrochloric acid solution (the recommended range is 6.95-7.05).

5. Make up to a net weight of 998-1002g with water and stir for 5-10 minutes. If there is a significant change in pH, continue to adjust the pH to the range of 6.95-7.05 using 5mol/L sodium hydroxide solution or 5mol/L hydrochloric acid solution.
6. Sterilize immediately by membrane filtration (pore size: 0.22μm).
7. Once the product is filtered, use immediately or store at 2 to 8°C for up to 12 months. Protect from light.

Cell Recovery

1. Cells transported on dry ice should be placed in a liquid nitrogen environment for 3-7 days before cell recovery.
2. Take 39mL of MetaCell® CHO-320 in advance and preheat it at 37 ℃ in a 125mL shake flask.
3. Remove 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 centrifuge tube containing 9 mL of pre-heated MetaCell® CHO-320.
5. Centrifuge at 1000rpm for 4 minutes, discard the supernatant, resuspend the cells in pre-heated MetaCell® CHO-320, and transfer all to a 125mL shake flask to make a final volume of 30mL. After mixing well, take a sample to measure the cell density and viability. The cell density should be within the range of 0.3-0.4 x 10^6 cells/mL.
6. Passage the cells when the cell density is ≥3.0 x 10^6 cells/mL and the viability is ≥90% after 3-4 days of culture. Note: If the cells have been revived for 2-3 generations and are showing normal growth with a viability of ≥95%, it is advisable to arrange for cryopreservation as soon as possible.

Cell Passaging

1. Pre-heat the culture medium at 37°C for 20-30 minutes or at room temperature for 1 hour before using it for subculturing.
2. Aseptically transfer the seed culture into a shaking flask and add an appropriate amount of culture medium. Set the shaker parameters according to the culture condition. Passage the cells every 3-4 days using fresh medium.
3. The recommended seeding density is:

   Passaging every 3 days: 0.3-0.5×10^6 cells/mL

   Passaging every 4 days: 0.15-0.3×10^6 cells/mL
4. Passaging can be performed when the final cell density reaches 4.0-6.0×10^6 cells/mL and the cell viability is ≥95%.
5. To achieve the best experimental results, cells should passage at least three times.
6. Transfection experiments should only be conducted after the cells have reached a stable growth state or have been adapted for about two weeks in the selection medium.

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 controlled at 10.0-15.0×10^6 cells/mL.
3. Cryopreservation solution: (90% MetaCell® CHO-320 + 10% DMSO), precool 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-320. 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-320.

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

• Direct Adaptation Method

For cells that can be directly adapted, when the cell viability is ≥95% and in the early logarithmic growth phase, try directly transferring from serum-free medium to MetaCell® CHO-320.

1. Inoculate CHO cells into fresh MetaCell® CHO-320 at a seeding density of 0.15-0.5×10^6 cells/mL (refer to the cell passaging steps).
2. 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 low, replace the adapted cells or use the indirect adaptation method.
3. Continue passage 3-4 times. When the cell viability is ≥95%, it can be considered that the cells have been adapted.

• Indirect Adaptation Method

1. Mix the original medium and MetaCell® CHO-320 at a volume ratio of 75:25, and the cell seeding density should be 0.15-0.5 ×10^6 cells/mL.

Product Name	Classification	Form	Cat No.	Size

2. Cells should be passaged when the cell density reaches 4.0-6.0×10^6 cells/mL after culturing for 3-4 days.
   (1) grow well and the viability is ≥90%, adjust the ratio of MetaCell® CHO-320 to the original medium to 50:50 during passaging.
   (2) If the cells grow slowly, cells can be subjected to centrifugation and media exchange, with centrifugation conditions at 1000rpm for 4 minutes. The mixed medium at this point still consists of MetaCell® CHO-320 and the original medium at a ratio of 25:75.
3. Repeat step 2, gradually increasing the proportion of MetaCell® CHO-320 (75:25, then 90:10), until the cells are completely transferred to 100% MetaCell® CHO-320.
4. Continue culturing in 100% MetaCell® CHO-320 for 3-4 passages. When the cell density reaches 4.0-6.0×10^6 cells/mL within 3-4 days of seeding and the cell viability is ≥95%, adaptation is considered complete. If the cell growth remains stable, subsequent experiments can be conducted.

Related Products

MetaCell® CHO-310	Basal Medium	Liquid	L1013-0500	500mL
			L1013-1000	1000mL
MetaCell® CHO TransFeed	Feed	Liquid	L1008-0500	500mL
			L1008-1000	1000mL
MetaCell® Titer Enhancer	BioReagent	Liquid	L1009-0010	10mL
			L1009-0100	100mL