MetaCell® HEK293-100 Chemically Defined Medium

User Manual

Product Description

MetaCell® 293-100 is a serum-free, chemically defined cell culture medium, optimized for the efficient transient transfection of HEK293 cells, especially 293F cells. This medium does not contain hydrolysates, proteins, or any animal derived components.

MetaCell® HEK293 -100 contains 4mM glutamine derivative.

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

Product Name	Cat No.	Size	Storage	Shelf Life	Application
MetaCell® HEK293-100	P2000-X010	10L	2-8°C, protected from light	12 months	Efficient transient transfection of HEK293 cells (protein or viral expression)
	P2000-X100	100L			

Cell Culture Conditions

Medium: : MetaCell® HEK293-100

Application: Suspension cell culture

Cell line: Expi293F™, FreeStyle™ 293-F, 293F, VPC2.0

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					

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 880-900g of ultrapure water or injectable water (water temperature 20-30°C) into a clean container.
2. Weigh out 23.280-23.374g of the MetaCell® HEK293-100 and slowly pour it into the container (labeled amount: 23.327g/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

8. Cells transported on dry ice should be placed in a liquid nitrogen environment for 3-7 days before cell recovery.
9. Take 39mL of MetaCell® HEK293-100 in advance and preheat it at 37 ℃ in a 125mL shake flask.
10. Remove a vial of frozen cells from the liquid nitrogen tank and thaw in a 37°C water bath (1-2 minute).
11. Transfer the cells to a centrifuge tube containing 9 mL of pre-heated MetaCell® HEK293-100.
12. Centrifuge at 1000rpm for 4 minutes, discard the supernatant, resuspend the cells in pre-heated MetaCell® HEK293-100, and transfer all to a 125mL shake flask to make a final volume of 30mL.
13. After 3-4 days of cultivation, subsequent experiments should be carried out when the cell density is ≥ 3.0 x 10^6 cells/mL and the viability is ≥ 90%.
14. We recommend to passage the culture for at least three passages before starting subsequent experiments.

Cell Passaging

1. Pre-heat MetaCell® HEK293-100 at 37°C for 20-30 minutes.
2. When the cell density reaches 3.0-4.0×10^6 cells/mL and the cell viability is ≥95% （day2-day4） , passaging culture can be performed.

Note: Different types of HEK293 cells 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 cell seeding density 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 2-4 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 controlled at 10.0-15.0×10^6 cells/mL.
3. Cryopreservation solution: (90% MetaCell® HEK293-100 + 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 HEK293 cells can be directly adapted to MetaCell® HEK293-100. If direct replacement of the medium (direct adaptation) fails, it is recommended to use gradient replacement (indirect adaptation) to adapt HEK293 cells to MetaCell® HEK293-100.

Note: HEK-293 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® HEK293-100.

1. Inoculate HEK293 cells into fresh MetaCell® HEK293-100 at a seeding density of 0.4-0.6×10^6 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 density reaches 3.0-4.0 x 10^6 cells/mL, and cell viability is ≥95%, it can be considered that the cells have been adapted.

• Indirect Adaptation Method

1. Mix the original medium and MetaCell® HEK293-100 at a volume ratio of 75:25, and the cell seeding density should be 0.4-0.6×10^6 cells/mL.
2. Cells should be passaged when the cell density reaches 3.0-4.0×10^6 cells/mL.
   (1) If the cells grow well and the viability is >90%, adjust the ratio of MetaCell® HEK293-100 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® HEK293-100 and the original medium at a ratio of 25:75.
3. Repeat step 2, gradually increasing the proportion of MetaCell® HEK293-100 (75:25, then 90:10), until the cells are completely transferred to 100% MetaCell® HEK293-100.
4. Continue culturing in 100% MetaCell® HEK293-100 for 3-5 passages. When the cell density reaches 3.0-4.0×10^6 cells/mL within 3-4 days of seeding and the cell viability is ≥95%, adaptation is considered complete.
5. Continue passaging at least 3 times. If the cell growth remains stable, subsequent experiments can be conducted.

Transfection

1. Before starting the cell transfection test, the cells should be fully adapted to MetaCell® HEK293-100, and passaged at the commonly used cell seeding density.
2. Inoculate the cells at a viable cell density of 5.0×10^5 cells/mL three days before transfection, or alternatively, inoculate the cells at a viable cell density of $2.0\text{-}2.5\times10^6$ cells/mL 18-24 hours prior to transfection.
3. On the day of transfection, the viable cell density should reach $4.0\text{-}6.0\times10^6$ cells/mL, and the cell viability should be ≥95% before proceeding with transfection.
4. For specific transfection procedures, please contact us.

Related Products

Product Name	Classification	Form	Product Code	Size
MetaCell® 293 TransFeed High Glucose	Feed	Liquid	L2009-0100	100mL
			L2009-1000	1000mL
MetaCell® PEI 40K	BioReagent	Liquid	L5001-0010	10mL
			L5001-0100	100mL