MetaCell® IncreaSect 900 Chemically Defined Medium

User Manual

Product Description

MetaCell® IncreaSect 900 is a serum-free insect cell culture medium designed to support high-density suspension cultures of Sf9 insect cells, specifically for use in baculovirus expression systems. The complete MetaCell® IncreaSect 900 medium consists of three components:MetaCell® IncreaSect 900, CD Lipid Concentrate A, and MetaCell® Glycerin, which are used in a ratio of 1 : 1 : 1000, respectively.

MetaCell® IncreaSect 900 contains 12mM 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® IncreaSect 900	P3200-X005	Powder	5L	2-8°C, protected from light	12 months	high-density suspension culture of Sf9 cells
	P3200-X050		50L			

Cell Culture Conditions

Basal medium: MetaCell® IncreaSect 900
Application: Suspension cell culture
Cell line: Sf9
Recommended set-up for initial trials:

Vessel volume	125mL	250mL	500mL
Medium volume	25-35	60-70	120-140
Shaker speed	125±5 rpm (amplitude 19mm) 120± 5 rpm (amplitude 25mm) 95± 5 rpm (amplitude 50mm)		
Types of flasks	PETG or PC, breathable, without baffles		
Culture environment	27 ± 0.5 ℃, 0% CO_2, no additional humidity is required. 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 920g of ultrapure water or water for injection (temperature at 20-30 °C) into a clean container.

2. Weigh out 41.808g-41.976 g of powder medium, slowly add it to the container and stir for 5 minutes until no lumps are present. The labeled amount of the medium is 41.892 g/L.
3. Add 8.0 mL of 5 mol/L sodium hydroxide solution and stir for 60 minutes until the solution has become clear.
4. Add 0.349-0.351 g of sodium bicarbonate to the solution, stir for 20 minutes until the sodium bicarbonate is completely dissolved. The final concentration of sodium bicarbonate should be 0.350g/L.
5. Adjust the pH to the desired range (recommended PH 6.20-6.30) using 5mol/L hydrochloric acid solution or 5mol/L sodium hydroxide 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 6.20-6.30 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. Remove a vial of cryopreserved cells from the liquid nitrogen storage tank and place it in a 37°C water bath for rapid thawing (1-2 minutes). Take out the vial when the ice block inside is almost completely melted.
3. Determine whether centrifugation is required upon the original characteristics of the cells:
   (1) If centrifugation is required: pre-heat 29 mL of MetaCell® IncreaSect 900 for over 30 minutes. Mix 9 mL of the pre-heated medium with the cell suspension in a centrifuge tube. Centrifuge at 200 × g for 5 minutes and discard the supernatant. Resuspend the cell pellet in the pre-heated MetaCell® IncreaSect 900. Transfer the entire resuspended solution to a 125 mL shake flask to achieve a final volume of 20 mL. Mix thoroughly and check cell density and viability; the target density should be within the range of 1.2-1.3 × 10^6 cells/mL.
   (2) If centrifugation is not required (e.g., for ExpiSf9™ Cells): Gently mix the cell suspension in the cryovial by pipetting. Transfer the entire cell suspension into a 125 mL shake flask containing pre-heated MetaCell® IncreaSect 900, ensuring a final culture volume of 20 mL.
4. Place the shake flask on a cell culture shaker with recommended parameters set at 27°C, 0% CO_2, and 95 ± 5 rpm (with an amplitude of 50 mm).
5. 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. Based on the cell count results, seed Sf9 cells at a density of 0.9-1.1 × 10^6 cells/mL and passage them after 3 days of culture. Ensure that the cells are passaged at least 3 times before proceeding with subsequent experiments.
2. Once the suspension culture stabilizes, cells should be cryopreserved as soon as possible. Typically, seeding SF9 cells at a density of 0.9-1.1 × 10^6 cells/mL results in a viable cell density of 5.0-7.0 × 10^6 cells/mL by day 3, with viability ≥95%.

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 25.0-28.0×10^6 cells/mL.
3. Pre-cool the cryopreservation solution (90% MetaCell® IncreaSect 900 + 10% DMSO) at 2-8°C for at least 30 minutes.
4. Take an appropriate amount of cell suspension, centrifuge at 200×g for 5 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 Sf9 cells can be directly adapted to MetaCell® IncreaSect 900. If direct replacement of the medium (direct adaptation) fails, it is recommended to use gradient replacement (indirect adaptation) to adapt Sf9 cells to MetaCell® IncreaSect 900.

Note: Sf9 cells used for adaptation need to be in the early logarithmic growth phase, with a cell viability≥90%.

• Direct Adaptation Method

For Sf9 cells that can be directly adapted, when the cell viability is ≥90% and in the early logarithmic growth phase, try directly transferring from the current serum-free medium to MetaCell® IncreaSect 900.

1. Inoculate the cells into fresh MetaCell®IncreaSect 900 at a seeding density of 0.9-1.1×10^6 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 ≥90%. If the viability is lower, use the indirect adaptation method described below.
3. Passage the cells for an additional 3-5 times, ensuring they are in good condition and growing normally, before proceeding with subsequent experiments.

• Indirect Adaptation Method

1. Mix the original medium and MetaCell® IncreaSect 900 at a volume ratio of 75:25, and the seeding cell density should be 0.9-1.1 ×10^6 cells/mL.
2. Cells should be passaged when the cell density reaches 5.0-7.0 ×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® IncreaSect 900 to the original medium to 50:50 during passaging.

(2) If the cells grow slowly, cells should be collected by centrifugation at 200×g for 5 minutes. Resuspended the cells in fresh mixed medium. The medium mix at this point still consists of MetaCell® IncreaSect 900 and the original medium at a ratio of 25:75.

3. Repeat step 2 and gradually increase the ratio of MetaCell® IncreaSect 900 (50:50, 75:25) until 100% MetaCell® IncreaSect 900 is used for cell culture.
4. Continue culturing the cells in 100% MetaCell® IncreaSect 900 for 2-3 passages. When the cell density reaches 5.0-7.0×10^6 cells/mL and the cell viability is ≥90%, 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.