MetaCell® Vero-200 Chemically Defined Medium

User Manual

Product Description

MetaCell® Vero-200 is a chemically defined, serum-free medium specifically designed for the adherent culture of Vero cells, supporting vaccine and virus production

MetaCell® Vero-200 contains no 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® Vero-200	P3001-X100	Powder	100L	2-8°C, protected from light	12 months	Adherent culture of Vero cells

Cell Culture Conditions

Basal medium: MetaCell® Vero-200
Application: Adherent cell culture
Cell line: Vero
Recommended set-up for initial trials:

Vessel volume	T25	T75	T175
Medium volume	30-35	60-70	120-140
Types of flasks	PS		
Culture environment	37 ± 0.5 ℃, 5% CO_2, humidity ≥80%, 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® Vero-200 contains no L-glutamine or its derivates. Please add L-glutamine or its derivates according to your needs.

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 15.400g-15.462 g of powder medium, slowly add it to the container and stir until no lumps are present. The labeled amount of the medium is 15.431 g/L.

3. Add 2.180-2.220g of sodium bicarbonate to the solution, stir until the sodium bicarbonate is completely dissolved. The final concentration of sodium bicarbonate should be 2.200g/L.
4. Adjust the pH to the desired range (recommended pH 7.20-7.40) using 5mol/L sodium hydroxide solution or 5mol/L hydrochloric acid solution.
5. 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 7.20-7.40 using 5mol/L sodium hydroxide solution or 5mol/L hydrochloric acid solution.
6. 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® Vero-200 at 37℃.
3. Take a vial of cryopreserved cells from the liquid nitrogen storage tank and thaw in a 37°C water bath for rapid thawing (1-2 minute). Take out the vial when the ice block inside is almost completely melted.
4. Transfer the cells to a 15 mL centrifuge tube containing 9 mL of pre-heated MetaCell® Vero-200.
5. Centrifuge at 100×g for 5 minutes. Discard the supernatant and resuspend the pelleted cells in pre-heated MetaCell® Vero-200 and transfer them to a T25 flask. Add MetaCell® Vero-200 to adjust the final volume to 4-6 mL.
6. When the cells have covered 80-100% of the surface area of the flask (1-3 days post-recovery), they can be passaged for subculture.
7. It is recommended to perform at least three passages before conducting subsequent experiments.

Cell Passaging

1. After 72 hours of cell culture, observe the cell growth using an inverted microscope. If the cells have reached approximately 90% confluency (typically within 3-5 days), they are ready for passaging.
2. Using a T25 flask as an example, discard the culture medium from the flask and rinse the monolayer of cells with 2 mL of pre-heated PBS solution (without Ca^{2+} or Mg^{2+} ions).
3. Add 1-2 mL of pre-heated trypsin solution (0.25% Trypsin-EDTA) to the flask, ensuring that all cells are exposed to the trypsin solution.
4. Discard the trypsin solution and incubate the flask in the incubator for 4-7 minutes, monitoring the cells under a microscope to check the digestion status.
5. When observed under the microscope, if all cells have become round-shaped and can be easily dislodged by gently tapping the flask, add 4-5 mL of pre-heated complete MetaCell® Vero-200. Use a pipette to gently detach the cells by pipetting up and down, thoroughly rinsing the cell surface.
6. Transfer the cell suspension to a 15 mL centrifuge tube and centrifuge at 100 × g for 5 minutes. Discard the supernatant and resuspend the cell pellet in 4-5 mL of pre-heated complete MetaCell® Vero-200. If cell clumping is observed, gently pipette or vortex to disperse the cells.

7. Count the resuspended cells using a cell counter and seed them at a density of 3.0-5.0 × 10^4 cells/cm^2 into new culture flasks.

Cell Cryopreservation

1. The final cell concentration for cryopreservation should be adjusted to 3.0-5.0×10^6 cells/mL.
2. Pre-cool the cryopreservation solution (92.5% MetaCell® Vero-200 + 7.5% DMSO) at 2-8°C for at least 30 minutes.
3. Take an appropriate amount of cell suspension, centrifuge at 100 × g for 5-10 minutes, discard the supernatant, and resuspend the cells in the pre-cooled cryopreservation solution.
4. Divide the cell suspension into cryotubes according to the cryopreservation specifications.
5. Gradually cool the cells to -80°C for freezing (cooling rate of 1°C/min) using a controlled-rate freezer or manual control method.
6. 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, Vero cells cultured in serum-free or low-serum (3% or less) conditions can be directly adapted to MetaCell® Vero-200. However, for Vero cells cultured with 5%-10% serum, a stepwise reduction in serum content is required. If direct replacement of the medium (direct adaptation) fails, it is recommended to use gradient replacement (indirect adaptation) to adapt Vero cells to MetaCell® Vero-200.

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

• Direct Adaptation Method

1. For Vero 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 or low-serum (3% or less) medium to MetaCell® Vero-200. For the first 2 to 3 passages, the seeding density should be twice the normal seeding density. Continue passaging the cells for an additional 3-5 times to ensure consistent cell growth. Once consistent growth is achieved, you can proceed with subsequent experiments.
2. For Vero cells cultured in 5-10% serum, if cell viability is ≥90% and they are in the early logarithmic growth phase, directly inoculate them into complete MetaCell®Vero-200 with 3% serum. After 2-3 passages, if cell condition and growth are normal, reduce the serum concentration to 1% for an additional 2-3 passages. Upon confirming normal cell state and growth again, transition the cells to serum-free complete MetaCell® Vero-200 and passage them 3-5 more times. Once consistent cell health and growth are verified, proceed with subsequent experiments.

• Indirect Adaptation Method

1. Mix the original medium and MetaCell® Vero-200 at a volume ratio of 75:25, and the seeding cell density should be twice the normal seeding density.
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® Vero-200 to the original medium to 50:50 during passaging.

(2) If the cells grow slowly, cells should be collected by centrifugation at 100 ×g for 5 minutes. Resuspended the cells in fresh mixed medium. The medium mix at this point still consists of MetaCell® Vero-200 and the original medium at a ratio of 25:75.

3. Repeat step 2 and gradually increase the ratio of MetaCell® Vero-200 (50:50, 75:25) until 100% MMetaCell® Vero-200 is used for cell culture.
4. Continue culturing the cells in 100% MetaCell® Vero-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. If the original culture contains a higher percentage of serum (5%-10%), a stepwise serum reduction protocol can be followed after indirect adaptation. For detailed steps, refer to the Step 2 of the direct adaptation method.
6. Continue the passaging for at least 3 times. If the cell growth remains stable, subsequent experiments can be conducted.