

Pesche-CD100S Vero Cell CD Medium

Product Name: Pesche-CD100S

User Manual

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Description

Pesche-CD100S Vero cell CD medium is a serum-free medium specially designed for virus production. It can support the high-density proliferation of Vero adhered cells, continuous generational culture and the production of a variety of viruses without adding serum.

Application

This product can be used in scientific research and large-scale production of biological drugs, but it cannot be directly used for human or medical purposes.

Composition

This medium contains:

- carbohydrates, amino acids, vitamins, metal ions, and other nutritional components.
- 3.5 g/L D-glucose, 0.3 g/L P188.
- Phenol red, HEPES, nucleoside.

Not contain:

- hydrolysates, antibiotics.
- Raw materials from animal sources.

Storage

- Store medium at 2-8°C, away from light.
- Once opened, the powder medium should be stored protected from moisture in a tightly sealed container.
- Do not use it after the expiration date or being damped.

Reconstitution of Powder Medium

Table 1 shows the preparation of Pesche-CD100S medium ^[1].

Ingredients	Concentration
Pesche-CD100S medium powder	15.00 g/L ^[2]
L-glutamine	0.584 g/L
Sodium bicarbonate	2.20 g/L

Table 1. Preparation of Pesche-CD100S medium

- 1) Weigh 90% water of the final volume into the preparation container using pure water, ultrapure water, or water for injection at 20-30°C. Mix thoroughly without creating air bubbles.
- 2) Accurately weigh the corresponding mass of Pesche-CD100S medium at a concentration of 15.00 g/L, and add it into the preparation container of 1) step.
- 3) weigh 0.584 g/L of L-glutamine powder, add it to the preparation container, and stir fully for 40 minutes until it is completely clear and transparent.
- 4) weigh 2.20 g/L sodium bicarbonate powder, slowly add it to the preparation container near the liquid surface, and stir for 3-5 minutes to dissolve.
- 5) Adjust to pH 7.1-7.3 with sodium hydroxide or hydrochloric acid solution if the pH is beyond this range.

- 6) Pass the medium solution through a pore size of 0.22 or 0.2 µm sterile filter membrane, such as PES, using a pulse pump or compressed air (3-15 psi).
- 7) Use the prepared medium liquid immediately or store it in glass bottles, PET storage bottles, or disposable storage bags with an oxygen barrier membrane in a dark environment of 2–8°C. It's recommended for use within one month.

Note:

^[1] The above parameters (such as stirring time) are set for small-scale liquid preparation. Adjust these parameters for large-scale preparation based on container capacity to ensure full dissolution of dry powder.

^[2] The "g/L" unit denotes volumetric concentration (solute mass/water volume).

Specifications of final liquid medium

Test	Unit	Specification
pH		7.1 – 7.3 ^[3]
Osmolality	mOsm/kg	280 – 340
Turbidity	NTU	< 4.00

Table 3. Specifications of final liquid medium

Note:

^[3] The pH buffer system of the product is carbon dioxide-sodium bicarbonate. The final pH value should be strictly controlled within the specific range outlined in Table 3. The following operations, such as prolonged reconstitution time or aeration in the bioreactor without pH control, can result in a gradual pH increase. There is a risk of metal ion precipitation when the pH value exceeds the upper limit.

Cryopreservation

- 1) Take the convergence degree of 80-90%, the activity rate is greater than 90%, and the sterile cells are frozen for microscopic examination.

Retain conditional culture medium (supernatant of medium).

- 2) The frozen medium is prepared with a ratio of 92.5% Pesche-CD100S medium (fresh medium and conditional medium 1:1) + 7.5% DMSO.
- 3) Cells use mild digestive enzymes (if you use ordinary trypsin, you need to use trypsin inhibitors to terminate digestion and centrifuge removal) to digest. After stopping digestion, 100xg is centrifugated for 5 minutes and discarded.
- 4) Use 2) prepared frozen storage medium to resuspend cells. After resuspension, the control of living cell density is 1-5×10⁶ cells/mL.
- 5) According to the specific needs of the project, step 4) the suspension will be stored in a freezing storage tube with suitable specifications.
- 6) Achieve cryopreservation in an automated or manual controlled rate freezing apparatus (0.5-1°C decrease per minute is suggested).
- 7) Transfer frozen cells to liquid nitrogen storage.

Cell Recovery

- 1) Rapidly thaw frozen cells in a 37°C water bath. Transfer to a clean workbench as soon as melted or with small ice crystals.
- 2) Transfer the cell suspension into a centrifuge tube containing 10-15 mL preheated Pesche-CD100S medium, centrifuge 100×g for 5 min, and discard the supernatant.
- 3) Use 15-20 mL preheated Pesche-CD100S medium to suspend cells and transfer to a T75 square bottle with a breathable lid.
- 4) Place the T75 square bottle in an incubator with 37°C, 5% CO₂ and saturated humidity.

Subculture Cells

- 1) The cell convergence degree is 80-90%, and the sterile cells are passed on by microscope.
- 2) Discard the culture solution, rinse twice with 10 mL (taking T75 as an example) PBS at room temperature, and discard PBS.
- 3) Add 1 mL (taking T75 as an example) mild digestive enzyme (if you use ordinary trypsin, you need to use trypsin inhibitors to terminate digestion and centrifuge removal), 37°C digest for 3-5 minutes until the cell begins to round and fall off.
- 4) Add the preheated Pesche-CD100S medium to contact all culture surfaces, terminate the digestion, and gently blow to collect the cells.
- 5) Sampling count, inoculation with $1-5 \times 10^4$ cells/cm², supplement Pesche-CD100S medium to 15-20 mL (take T75 as an example).
- 6) Placed in an incubator of 37°C, 5% CO₂ and saturated humidity. Generally, the convergence degree is 80-100% in 2-5 days.

Related Product

Product Name	Cat. No.	Form	Size	Packaging	Note
Pesche-CD100S Vero Cell CD Medium	EXP0117501	Powder	200 L	Bag	No serum, ultra-low protein, no animal source, clear chemical composition, support the production of COVID-19 vaccine, rabies vaccine, rotavirus vaccine, etc.
	EXP0117502	Powder	100 L	Bag	
	EXP0117503	Powder	10 L	Bottle	
Pesche-CD100 Vero Cell CD Medium	EXP0117504	Liquid	1 L	Bottle	

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