

Product Instruction

SF501 LMH Serum-free Medium

Product Type: SF501

Product Description

LMH serum-free medium is a personalized serum-free medium, which is independently developed, researched and produced by Shanghai BioEngine Sci-Tech Co., Ltd. This medium is suitable for high-density suspension culture of LMH cells and high-efficiency expression and production of adenovirus.

Product Formula

The intellectual property rights of LMH Cell serum-free medium formula are owned by Shanghai BioEngine Sci-Tech Co., Ltd. For additional information, please contact our technical support department.

Product Ingredient

The medium contains carbohydrates, amino acids, vitamins, metal ions and other nutritional components.

This product does not contain components of animal origin, genetically modified plant origin or raw material with mad cow virus origin.

This product does not add any hormones, antibiotics, organic solvents and preservatives.

Product Preservation

- Store in a dark environment at 2-8°C.
- This product is vulnerable to water damage. Please use immediately after opening. If it needs to be stored, please sealed by heat sealing and sealing clips, avoiding damp and being ineffective.
- Do not recommend to use, when the product is beyond expiration date.

Instruction for the preparation of LMH medium

Do the preparation of medium as per the one that the following table shows

Component	Concentration
Dry powder of LMH serum-free medium	23.30 g/L
Sodium hydroxide	0.24 g/L
Sodium bicarbonate	2.00 g/L

(1) Weigh 100% water of the final medium preparation volume into the medium preparation container. Purified water or water for injection and above standard water should be used for preparation, and the water temperature should be controlled at 20-30°C.

(2) Turn on the mixing system of the medium preparation container, stir thoroughly, and avoid the generation of air bubbles during stirring.

(3) Accurately weigh 23.30 g/L of medium dry powder, add them into the preparation container near the liquid surface or use special equipment such as homogenizer, and stir thoroughly for 15-25 min.

(4) According to the dosage of 0.24 g/L, slowly add sodium hydroxide particles to the solution prepared in step (4), and stir thoroughly for 13-17 min.

(5) Accurately weigh 2.00 g/L sodium bicarbonate powder, and add them into the preparation container near the liquid surface or use special equipment such as homogenizer, and stir thoroughly for 13-17 min.

(6) Use 1 mol/L hydrochloric acid solution to adjust the pH value of the medium to 7.0-7.4 (If necessary).

(7) It is recommended to use a pulse pump or compressed air (3-15 psi) to sterile filter the medium solution through a sterile filter membrane with 0.22 µm pore size.

(8) The prepared medium liquid should be stored in a dark environment at 2-8 °C, and the expiration date is one month.

(9) The reference parameters of product

Indicator	Reference Standard
Product initial pH value	3.70-4.40
Osmolality	280-340 mOsm/Kg
Product turbidity	<2.00NTU

Notes:

(1) The above units of "g/L" are volume concentration (solute weight/solution volume).

(2) The above preparation parameters (such as stirring time, etc.) are for the reference of small-scale preparation in research and development. When in production, please set

appropriate preparation parameters according to the stirring capacity of the preparation vessel.

(3) The product belongs to carbon dioxide buffer system. The product final pH value may rise when vigorous stirring or long-time stirring, which is a normal phenomenon and does not affect the use of the product.

Medium usage

Subculture

- The seeding density should be controlled at $0.8-1.2 \times 10^6$ cells/ml during serum-free suspension subculture, and subculture should be carried out every 48 hours.
- Use the shake flask with a vented cap and place it in a 37°C, 5% CO₂ environment. The recommended shaker speed is 110-130 rpm.

Cryopreservation

Select the cells that are in good condition in the logarithmic growth phase for cryopreservation. The cryopreservation density is $2.5-3.5 \times 10^7$ cells/ml/tube. The ratio of cryopreservation solution is 93% fresh medium + 7% DMSO. Centrifuge 190g cells for 5 min, discard the supernatant, resuspend the mixed freezing solution, and dispense the cells into freezing tube, 1 ml/vial. Then, place them in a programmed cooling box at -80°C overnight, and transfer to liquid nitrogen save

Recovery

Rotate the freezing tubes in the same direction in a 37°C water bath to quickly melt the cryopreservation solution, and take them out to a clean bench when only small ice crystals remain. Add 10ml of culture medium and 190g cell to the centrifuge tube, centrifuge for 5 min, and wash away the DMSO. Use 20-30 ml of medium to resuspend the cells into a 125 ml shake flask with a vented cap, and control the seeding density at $0.8-1.2 \times 10^6$ cells/ml.