Celkey® AM LSM 598

Low Serum Medium

Catalog No.: 22108-598

For scientific research and production, used for tissue and cell culture in vitro, shall not be used for human and animal clinical diagnosis and treatment.

Product Descriptions [1]

Celhappy® AM LSM 598 is a chemically defined medium that supports Vero, Marc-145, BHK21, MDCK, MDBK, PK15, HEK293, ST and other adherent cells to grow with high density and low serum while supporting serum-free production. Cells are subcultured and grown. The amount of serum in the phase can be reduced to between 1.0 - 5.0%, and the maintenance phase after inoculation can achieve serum-free maintenance, which extends cell viability, shortens cell doubling time, and reduces production and purification costs. AM LSM 598 medium can be used in every stage without serum of the passage and scale-up of adherent cells in T-flasks, cell factory, roller bottle, spinner and microcarrier processes, as well as virus production.

Product Name	Product No.	Specification	Form	Storage	Shelf Life
AM LSM 598	22108-598	5 L/ 10 L/ 50 L/ 100 L/ 500 L/ Customized	Dry Powder	2-8℃ Protect from light	24 Months

[1] Each product can be customized according to your needs: Package Size & Format, Form (Dry Powder or Liquid).

Shelf life duration is determined form date of manufacture, additional reagents may affect the storage conditions and shelf life of the medium.

Formulation [2]

With

- 4.5 g/L Glucose
- 4.0 mM L-Glutamine
- Pyruvic Sodium

Without

- Phenol Red
- Sodium Bicarbonate

[2] AM LSM 598 medium is the commercial formulation independently developed by JSBio. For more information, please contact us at the end of this article.

Preparation Instructions [3]

1. Fill the mixing container with purified water (20 - 30℃) at 80% - 90% of the final volume.
2. Slowly add 13.71 g/L of dry powder medium with gentle stirring. Mix for 30 minutes.
3. Slowly add 2.0 g/L of sodium bicarbonate (CAS: 144-55-8). Mix for 10 minutes.
4. Adjust the pH to 6.8 - 7.0 using 1 M - 5 M NaOH or HCl. The pH may rise 0.1 to 0.2 after sterile filtration.
5. Adjust the final volume with purified water. Mix for 10 minutes.
6. Filter the media using a membrane filter with 0.22 μm pore size immediately.

[3] Avoid repeated prewarming of AM LSM 598 liquid medium.

For component customization requirements, please contact us at the end of this article.

Culture Conditions

Medium: Celkey® AM LSM 598

Cell Line: Vero, Marc-145, BHK21, MDCK, MDBK, PK15, HEK293, ST and other adherent cells

Serum concentration: 1.0% - 5.0% during growth period (passage), during production period (after poisoning), serum-free

Inoculation density: see the recommended values in the table below

Culture type: Adherent

Culture vessels: T-flasks, roller bottle, spinner and 3 L bioreactor

Recommended parameters for different culture vessels:

T-flasks:	
Items	Recommended Parameters
T-25 cm^2 flasks	Culture Volume: 8 - 10 mL Seeding Density: 0.5 - 1.0 x 10^6 cells/T25 Seeding ratio: 1:4 - 1:8

T-75 cm^2 flasks	Culture Volume: 15 - 30 mL Seeding Density: 2.0 - 4.0 x 10^6 cells/T75 Seeding ratio: 1:4 - 1:8
T-175 cm^2 flasks	Culture Volume: 40 - 50 mL Seeding Density: 4.0 - 6.0 x 10^6 cells/T175 Seeding ratio: 1:4 - 1:8
T-225 cm^2 flasks	Culture Volume: 50 - 70 mL Seeding Density: 6.0 - 8.0 x 10^6 cells/T225 Seeding ratio: 1:4 - 1:8
Culture Temperature	37℃
CO_2 Concentration	5%
Relative Humidity	60% - 80% RH

Spinner (Microcarrier):

Items	Recommended Parameters
100 mL- Spinner	Culture Volume: 50 - 100 mL
250 mL- Spinner	Culture Volume: 10 - 200 mL
500 mL- Spinner	Culture Volume: 200 - 400 mL
1000 mL- Spinner	Culture Volume: 400 - 800 mL
Microcarrier Type	Cytodex 1
Microcarrier Concentration	3 - 10 g/L
Seeding Density	0.2 - 0.5 x 10^6 cells/mL
Shaking Speed	30 - 50 rpm
Culture Temperature	37℃
CO_2 Concentration	5%
Relative Humidity	60% - 80% RH

3 L Bioreactor [4]

Items	Recommended Parameters
Shaking Speed	40 - 60 rpm Adjust this value according to the bioreactor diameter-to-pitch ratio of different manufacturers.
Culture Temperature	37℃
CO_2 Sparger	0.01 VVM
Air Sparger	0.01 VVM
O_2 Sparger	The initial setting is 0.02 VVM It should be upregulated according to the increase of viable cell density.
pH	7.1 ± 0.2
DO	30% - 50%

[4] An additional 1.0 g/L Pluronic F-68 is recommended to protect cells from shear force during bioreactor culture.

Additional anti-clashing reagents is recommended to prevent cell clumping during "ball-to-ball" culture of microcarriers.

Recover Cells

The cryopreserved volume is 1.0 mL/ tube, the cryopreserved density is 5.0 x 10^6 cells/mL, and the cells are recovered into T-75 cm^2 flasks for adherent culture as an example.

1. Remove the cryopreserved tube from the liquid nitrogen, rapid thaw (＜1 minute) the frozen cells in 37℃ water bath, avoid immersing the lid of cryopreserved tube into the water.
2. Transfer all cell fluid into a sterile centrifuge tube with 20 mL prewarmed 1.0 - 5.0% serum medium, centrifuge the cells at 150 - 300 g (about 800 - 1150 rpm) for 5 minutes, aspirate and discard the supernatant, then resuspend cells with 30 mL fresh prewarmed 1.0 - 5.0% serum medium and transfer them into a new sterile T-75 cm^2 flasks. Observe the cells under a microscope.
3. Passage the cells every 72-96 hour, and the method should refer to the steps of "Cell Passage and Scale-up". And the other experiments should be carried out after 3 - 5 continuous passages

Cell Passage and scale-up

The cells which are cryopreserved in AM LSM 598 medium after recover, or the cells which have adapted to AM LSM 598 medium can be conducted continuous passage directly and scale-up the cells according to the needs of subsequent experiments. The steps of cell passage and scale-up are as follows:

1. In the culture container, the cells were covered with a single layer, discard the liquid, then discard the PBS (1x, without Ca^{2+}, without Mg^{2+}) after cleaning the cells once with PBS.
2. Add proper amount of 0.25% Trypsin-EDTA (1X) to completely cover the bottom of the culture container, place at 37℃ for digestion for 1 - 3 minutes. Then discard the digestion solution and tap the bottom of the culture container to dissociate the cells completely .
3. Add proper amount of 1.0 - 5.0% serum medium to stop digestion, gently blow the cells away to form cell suspensions.
4. Determine viable cell density (x 10^6 cells/mL) and viability (%) using cell counter equipment.
5. If the T-75 cm^2 square bottle is inoculated with a density of 2.0 - 4.0 x 10^6 cells or a passage ratio of 1:4 - 1:8, calculate[5] required volume of AM LSM 598 medium, serum and the cell fluid according to the recommended seeding density and seeding ratio, conduct cell passage and scale-up directly.
6. During the first passage to the second passage, it is recommended that the seeding density or seeding ratio is twice as much as that of normal passage.
7. Before each passage, discard the supernatant of the cultures directly if adherent cell confluence＜90%, resuspend the cells with 100% fresh prewarmed AM LSM 598 medium equal to the working volume. Otherwise, conduct cell passage and scale-up directly according to Step 2 of “Cell Passage and Scale-up”.
8. Culture the cells referring to the “Culture Conditions”, passage the cells every 72 ± 4 hour. And the other experiments should be carried out after 3 - 5 continuous passages.

[5] Calculated formula:

Required volume of cell fluid (mL) = Seeding density (x 10^6 cells/mL) x Working volume (mL) ÷ Viable cell density before passage (x 10^6 cells/mL).

Required volume of AM LSM 598 medium (mL) = Working volume (mL) – Required volume of cell fluid (mL).

Required serum volume (mL) = working volume (mL) x serum concentration (%).

Cryopreserve Cells

Cryopreservation is recommended for cells in mid-log phase of growth with adherent cell confluence＞90%.

1. Put the freezing container with fresh isopropanol, the cryopreserved tubes and other required materials into 2 - 8℃ for pre-cooling for 24 hours or -20℃ for pre-cooling for 2 - 4 hours in advance.
2. Prepare the fresh cryopreservation solution, calculate [6] the required volume of culture medium, serum and DMSO, mix and put it on ice or pre-cool at 2 - 8℃, please make it as needed.
3. In the culture container, the cells were covered with a single layer, discard the liquid, then discard the PBS (1x, without Ca^{2+}, without Mg^{2+}) after cleaning the cells once with PBS.
4. Add proper amount of 0.25% Trypsin-EDTA (1X) to completely cover the bottom of the culture container, place at 37℃ for digestion for 1 - 3 minutes. Then discard the digestion solution and tap the bottom of the culture container to dissociate the cells completely .
5. Add proper amount of 1.0 - 5.0% serum medium to stop digestion, gently blow the cells away to form cell suspensions. Determine viable cell density (x 10^6 cells/mL) and viability (%) using cell counter equipment.
6. The cryopreserved volume is 1.0 mL/ tube, the cryopreserved density is 5.0 - 10.0 x 10^6 cells/mL. calculate [6] required volume of AM LSM 598 medium for resuspension, the required volume of the cell fluid, and required volume of cryopreservation solution.
7. Sample the cell fluid according to the calculation results, centrifuge the cells at 150 - 300 g (about 800 - 1150 rpm) for 5 minutes, aspirate and discard the supernatant, then gently pat the bottom of the centrifuge tube to make the cell precipitation evenly dispersed. Add the required AM LSM 598 medium for resuspension according to the calculation results and prepare the cell suspension.
8. Add the pre-cooled cryopreservation solution drop by drop into the cell suspension to prepare the cryopreserved cell suspension, and gently shake while adding to ensure the uniformity of cells. This step needs to be carried out in the ice bath.
9. Place the pre-cooled cryopreservation tubes on the ice, and the cryopreserved cell suspension are divided into 1 mL of volume for each tube. During the loading period, the cryopreservation cell suspensions were mixed with light shaking repeatedly.
10. After the separation, transfer the cryopreserved tubes to the pre-cooled freezing container, store it in the -

80℃ refrigerator for at least 24 hours, and transfer it to the liquid nitrogen tank for long-term storage.

[6] Calculated formula:

Required total number of cells (x 10^6 cells/mL) = Cryopreserved density (x 10^6 cells/mL) x Required number of cryopreserved tubes (tubes).

Required volume of cell fluid (mL) = Required total number of cells (x 10^6 cells/mL) ÷ The determined viable cell density (x 10^6 cells/mL).

Required volume of cryopreserved cell suspension (mL) = Cryopreserved volume/ tubes (mL) x Required number of cryopreserved tubes (tubes) = Required volume of cell suspension (mL) + required volume of cryopreservation solution (mL).

Required volume of cell suspension (mL) = Required volume of cryopreservation solution (mL) = Required volume of AM LSM 598 medium for resuspension (mL).

Required volume of cryopreservation solution (mL) = 10% DMSO (mL) + 50% AM LSM 598 medium (mL) + 40% Serum (mL).

Related Products

Product name	Catalog No.
0.25 % Trypsin-EDTA	99008-14020

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