User's Manual - MCM CuLMSC-SFM31(A)

【Product Name】: MSCs serum-free culture medium

【Product Model】: MCM CuLMSC-SFM31(A)

【Product Number】: CM031(A)-500

【Packing Specifications】: 500 mL / bottle;

【Main Component】: MCM CuLMSC-SFM31(A) is Serum-free, Xeno-free medium. It mainly contains glucose, amino acids, inorganic salts, trace elements, cytokines and growth factors etc.

【Intended Use】: In vitro culture of serum-free expansion culture of human mesenchymal stem cells (derived from umbilical cord, placenta, fat, etc.).

【Performance Index】:

Sterility: Negative (bacteria, fungi)	Endotoxin: ＜ 1EU/mL
pH: 7.0 ~ 7.6 (RT)	Osmolality: 280 ~ 350 mOsm/kg
Appearance: Colorless to light yellow ,clear liquid	

【Delivery Requirements】:

MSCs serum-free medium: -30 ~ -5℃, protected from light.

【Storage Conditions and Validity Period】:

MSCs serum-free medium: -30 ~ -5℃, protected from light. The validity period is 12 months.

【Usage Method】: In order to maximize the performance of the culture medium, Please refer to the following instructions: Thaw the frozen products in a 37°C water bath before use, and remove it immediately after thawing. Thawing at 4°C or room temperature is not recommended as it may increase sediment or insoluble particle formation.The thawed and unopened MSCs serum-free medium could be stored at 2 ~ 8℃ for 1 month.

I.Operating Procedures (take 75T culture flask as an example)

1.Primary Cell Culture

1.1.Tissue explant

1.1.1.Pre-warmed MCM CuLMSC-SFM31(A) in a 37°C incubator.

1.1.2.Operate according to the tissue explant method. And then transplant the tissue blocks into the culture carrier according to the corresponding operation requirements.

1.1.3.According to the corresponding the operating procedures, add a certain amount of MCM CuLMSC-SFM31(A) and culture at 37°C, 5% CO_2 and 85% humidity condition.

1.1.4.Observe and change the fluid according to the corresponding operation requirements. When the cell confluence reaches more than 80%, digest and collect the cells.

1.2.Trypsinization culture method

1.2.1.Pre-warmed MCM CuLMSC-SFM31(A) in a 37°C incubator.

1.2.2.Operate according to the enzymatic digestion method to obtain primary cell suspension, cell seeding a certain density (20000/cm^2 recommended), add 15 ~ 20mL MCM CuL MSC-SFM31(A), and place it at 37°C, 5 % CO_2 and 85% humidity conditions.

1.2.3.Change the medium every 3 days, and digest and collect the cells in the flask when the confluency of cells in the flask reached more than 80%.

2.Digestion and Cell Passaging

2.1.Microscopic observation, digested and passaged the cells when the cell confluence reaches more than 80%.

2.2.Pre-warmed MCM CuLMSC-SFM31(A) in 37°C incubator.

2.3.Discard the medium in the culture vessel with a pipette, add an appropriate amount of DPBS, blow gently and discard, repeat once.

2.4.Add 5mL of trypsin or trypsin substitute (1×) and shake it evenly, digest at 37°C or room temperature. During digestion, the cells are observed with a microscope to see if they are rounded. Tap the side of Flask several times to detach cells, and observe whether the cells are suspended under the microscope. This step must be completed as soon as possible, no more than 5 minutes.

2.5.Add MCM CuL MSC-SFM31(A) to stop digestion and transfer the cell suspension to a 50mL centrifuge tube.

2.6.Centrifuge at 300g for 5min, discard the supernatant, add 10 mL of MCM CuLMSC-SFM31(A) to resuspend, sampling and cell counting, and centrifuge the cell suspension again.

2.7.After centrifugation, remove the supernatant, and add an appropriate volume of MCM CuLMSC-SFM31(A) to resuspend.

2.8.According to the counting results, cell seeding at a certain density (6000 ~ 12000/cm^2 recommended), add 15 ~ 20mL MCM CuLMSC-SFM31(A), and culture at 37°C, 5% CO_2 and 85% humidity conditions.

2.9.Change the medium every 3 days, and digest and collect the cells when the cell confluence in the flask reached more than 80%.

3.Cryopreservation and Thawing

3.1.Cell cryopreservation

3.1.1.Microscopic observation, when the cell confluence reaches more than 80%, digesting and collecting MSCs.

3.1.2.After centrifugation, resuspend the cell suspension with a certain amount of DPBS, perform cell counting and activity testing, and centrifuge the cell suspension again.

3.1.3.After centrifugation, remove the supernatant, and slowly add a certain amount of cell preservation solution to the cell pellet according to the results of cell count and activity testing, and gently mix by pipet tube.

3.1.4. Operate according to cryoprotectant user's manual.

3.2.Thawing

3.2.1.The operator shall wear anti-freezing protective equipment, take out the MSCs from the storage location quickly, immediately put them into the 37°C water bath / dry bath recovery equipment for thawing.

3.2.2.Transfer MSCs suspension to 15/50mL centrifuge tube, slowing add MCM CuLMSC-SFM31(A) (1:10 or higher) and mix gently.

3.2.3.Centrifuge at 300g for 5min and remove supernatant.

3.2.4.Add an appropriate volume of MCM CuLMSC-SFM31(A) to resuspend, sampling and cell counting.

3.2.5.According to the cell counting results, seeding at a certain density (recommended 6000 ~ 12000/cm²) into culture flasks, add 15 ~ 20mL MCM CuLMSC-SFM31(A), and place at 37°C, 5% CO_2 and saturated humidity conditions for culture.

3.2.6.Change the medium every 3 days, and digest and collect the cells when the cell confluence in the flask reached more than 80%.

【Suggestions】:It is recommended to pre-warmed at room temperature or 37°C before use.

【Precautions】:

1.This product is only available for scientific research and is not available for clinical treatment.

2.The product shall be used or subpackaged immediately after opening, otherwise the performance of the product will be affected.

3.It is recomend to conduct pre-experiment first.

4.The waste disposal shall comply with the relevant laws and regulations of the location of the user.

【Date of Instruction Manual Approval and Modification】: 2024.05

【Technical Support】: According to the terms of sales, please contact our technicians with any problems :

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