AMMS ® MSC Kit 2.0 (Phenol Red Free) Instructions

(No xenobiotic ingredients)

Product Name

English Name:AMMS ® MSC Kit 2.0 (without phenol red)

Product Performance

Catalog Number: AS-33

Set composition:

Component Name	Cat. No.	Specification	Amount	Storage conditions	Product Characteristics	Shelf Life
AMMS ® MSC Supplement 2.0	AS13 -1	25mL	1 bottle	-20 ℃, Protect from light	liquid	18months
AMMS ® MSC Basal Medium	AS09-11	500mL	1 bottle	2-8 ℃, Protect from light	liquid	18months

Product Description

AMMS®MSC Basal Medium (Phenol Red Free) is a standardized, xeno-free, serum-free medium for culturing human mesenchymal stem cells and their primary progenitor cells (MSCs). AMMS ® MSC Cell Culture Kit 2.0 (Phenol Red Free) supports the long-term growth of MSCs and maintains their multipotent differentiation potential.

Features:

1. Suitable for primary and subculture culture of human mesenchymal stem cells from different sources ;

2. Maintain the phenotype and multidirectional differentiation characteristics of human MSCs while maintaining their rapid expansion;

3. The endotoxin content of complete culture medium is less than 0.25 EU/mL;

4. Serum-free culture medium and additives contain no xenobiotic components and no antibiotics ;

5. The culture medium contains glutamine, so there is no need to add glutamine during the culture process .

Instructions for Use

Preparation of MSC complete medium :

1. AMMS®Place MSC Supplement 2.0 in a 37℃water bath until completely melted and then take it out immediately . If a small amount of flocs or turbidity appears, it is normal and will not affect the cell culture performance. It is recommended to remove the flocs by centrifugation (3400g, 3-5min) and use it directly or store it in aliquots at -20℃to avoid repeated freezing and thawing .

2.Add 25 mL of AMMS ®MSC Supplement 2.0 to 500 mL of AMMS ® MSC Basal Medium (without phenol red) and mix thoroughly .

Primary cell culture (taking umbilical cord as an example) :

1. Umbilical cord washing : Use sterile toothed forceps to transfer the umbilical cord to a 10 cm sterile culture dish, disinfect the entire outer surface of the umbilical cord with medical iodine, transfer it to a new dish, add 75% ethanol to immerse the entire umbilical cord, disinfect and sterilize it, then transfer it to a new dish, add 5 to 10 ml of sodium chloride injection to rinse, repeat 2 to 3 times to remove blood stains.

2. Use sterile tissue scissors to cut the umbilical cord into several sections of about 2 to 3 cm . Add 5 to 10 ml of sodium chloride injection to wash the blood clots. Repeat the washing until the blood stains are basically removed and the washing solution is clear.

3. Remove blood vessels: Remove two arteries and one vein of the umbilical cord in a spiral pattern.

4. Separate Wharton's jelly: Tear off the Wharton 's jelly with toothed tweezers , put it into a sterile dish, add 5-10 ml of sodium chloride injection solution, and wash the colloid.

5. Washing colloid: Transfer the obtained colloid to a 50ml centrifuge tube, add 20-30ml of sodium chloride injection, and shake gently at 2000rpm for 5min .

6. Colloid weighing: discard the supernatant and record the weighing.

7. Tissue homogenate: Add 2-3 ml culture medium to the colloid, use sterile tissue scissors to cut the tissue into tissue homogenate blocks in a centrifuge tube, centrifuge at 2000 rpm for 5 min, and remove the supernatant.

8. According to the weight of the colloid, add appropriate amount of complete culture medium, make up to volume, pipette evenly, inoculate into culture bottles (T75 bottles) at a rate of 0.5 g per bottle , and culture in 8 - 10 ml of culture medium (T75 bottle) per bottle .

9. Place the culture bottle flat so that the tissue blocks are evenly distributed on the entire bottom surface, and place the culture bottle in a carbon dioxide constant temperature and humidity incubator. Culture conditions: 37± 0.5℃, carbon dioxide volume fraction 5± 0.2 % .

10. On the 7th day of culture, replace the medium completely, collect the tissue blocks , and discard the supernatant after centrifugation . Resuspend the tissue blocks in fresh complete medium and evenly distribute them in the flask .

11. The cells can be seen in 5-9 days and can be harvested in 10-14 days.

12. When the degree of cell fusion near more than 3 tissue blocks reaches more than 85%, the cells can be harvested, the culture supernatant is poured out , the bottom of the culture bottle is washed 1 to 2 times with physiological saline , and the cells are digested with 0.05% trypsin or recombinant cell digestion solution . After terminating the digestion, the cell suspension is collected into a centrifuge tube, the cell fluid is passed through a cell sieve to remove the tissue, and centrifuged at 450g for 5 minutes. The precipitate is the primary harvested cells.

MSC cell culture :

1. Take the resuscitated or subcultured MSCs and count them.

2. Resuspend the cells with the prepared complete medium and culture them in flasks according to the flask density : 4000-6000 cells/cm2 for P1-P3 generations , 6000-8000 cells /cm2 for P3 and above generations, flask volume: 5 -10mL per flask for T25 flasks , 10-15 mL per flask for T75 flasks, 30 mL per flas for T175 flasks. After cell flasks are plated , shake well and place in a 37℃ carbon dioxide incubator for culture.

3. After 72 hours of cell culture, observe that the cell fusion degree reaches more than 85%. Pour out the culture supernatant , wash the bottom of the culture bottle with physiological saline 1-2 times , use 0.05% trypsin or recombinant cell digestion solution to digest the cells . After terminating the digestion, collect the cell suspension into a centrifuge tube, centrifuge at 450g for 5 minutes, and the precipitate is the harvested cells.

Precautions

1. AMMS® MSC Supplement 2.0 was rethawed in a 37℃ water bath and used directly after rethaw or stored in aliquots at -20°C to avoid repeated freezing and thawing .

2. AMMS® After reconstitution of MSC Supplement 2.0, a small amount of flocs or turbidity may appear, which is normal and will not affect the cell culture performance. It is recommended to remove the flocs by centrifugation (3400g, 3-5min) .

3. AMMS® MSC Supplement 2.0 can be stored at -20°C to -40°C until the expiration date. Once thawed, it can be kept at 4°C for up to 7 days . The prepared complete medium is stable at 4 degrees for14 days.

References

1. S. Gottipamula, MS Muttigi, U. Kolkundkar and RN Seetharam , Serum-free media for the production of human mesenchymal stromal cells: a review , Cell Prolif, 6 (12) (2013) : 608–627.

2. Chi-Hsien Liu, Mei-Ling Wu b, Shiaw-Min Hwang , Optimization of serum free medium for cord blood mesenchymal stem cells , Biochemical Engineering Journal 33 (2007) 1–98.