Santeja+

Ultra ExoM™ Culture Medium for Extracellular Vesicles (EVs)

Article Number: FK-K0204024

Santeja Inc

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Culture Medium for Extracellular Vesicles (EVs)

Product information

Product Name	**Ultra ExoM™ Culture Medium for Extracellular Vesicles (EVs)**
Article Number	**FK-K0204024**
Specification	Basal medium **450mL**, supplement **50mL**
Storage	**2-8℃**
Application	Mesenchymal stem cells derived from human bone marrow, fat and umbilical cord Primary isolation, amplification and subculture
Shelf life	12 months

Product Brief

Ultra ExoM™ Culture Medium for Extracellular Vesicles (EVs) is a human mesenchymal stem cell basal medium independently developed by **Santeja Inc**, which has no external animal ingredients, serum-free, no need separate trophoblast cells, clear chemical composition. It can be used alone without mixing with basal cell culture medium. It can be applied to the primary isolation, expansion and subculture of mesenchymal stem cells from human bone marrow, fat, umbilical cord and other tissue sources, and maintain their multi-directional differentiation potential. The endotoxin level of this product is far lower than the standard of Chinese Pharmacopoeia. The production process follows ISO9001 system and GMP level producing workshop, strictly follow cGMP operating standards, each index is superior to the industry standard.

Culture Medium for Extracellular Vesicles (EVs) main ingredients: amino acids, vitamins, inorganic salts, albumin, transferrin, insulin, trace elements, cytokines, etc.

Product features

- There is no foreign animal protein component, which greatly reduces the risk of pollution of various viruses, molds and mycoplasma.
- No serum was produced in the whole process, which greatly reduced the difference between batches.
- It can be used for primary generation separation, and the culture process does

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not need to cover the culture plate.

- Used alone, no need to mix with basic cell culture medium
- The efficiency of amplification was high, the proliferation doubled around 24 hours, and the culture time was saved.
- Endotoxin < 0.06eu/ml, far lower than the level of Chinese Pharmacopoeia

Product content

Content	Specifications	Quantity	Transport
Basal Medium	450 mL	1 bottle	Ice bag 2~4℃
Supplement additive	50 mL	1 vial	Ice bag -20~-80℃

Related products

Exosomes RNA Extraction Kit (Product code：FK-K0102001)
Xeno free cell digestive solution (fk-k0204009)
Proper serum free cell cryopreservation solution (fk-k0204005)

Experimental preparation

Preparation of Culture Medium for Extracellular Vesicles (EVs)

1.1 When the culture supplement additive was thawed rapidly at 37℃, it was not easy to destroy the nutrients in the supplement additive, and the time was about 10 minutes. After the supplement additives are melted, shake them well, then pack them separately or add them directly to the basic culture medium in proportion. After the subpack, the additives are immediately stored at-20℃to -80℃, avoiding repeated freezing and thawing.

1.2 Add Culture supplement additive (50mL) into basal medium (450mL) in the ratio 1:9, mix well. The mixed medium is Extracellular Vesicles (EVs) culture medium, which can be stored at 2-8℃ for 2 – 3 weeks. It is not recommended to use the medium which had been prepared for more than 3 weeks. The basal medium and the medium additives can be stored for more than 1 year before being mixed. Culture Medium for Extracellular Vesicles (EVs) can be directly used for cell culture. No need to mix with another cell culture medium.

1.3 Culture Medium for Extracellular Vesicles (EVs) can be directly applied to the primary isolation, expansion and subculture of mesenchymal stem cells from human bone marrow, fat, umbilical cord and other tissues.

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Operation method(The following steps should be carried out under sterile conditions)

HMSC cell resuscitation (10cm dish)

2.1 The cryopreserved hMSC cells were removed from liquid nitrogen, and the cryopreserved tubes were quickly put into a 37 ℃ water bath for rapid dissolution.

2.2 In the biosafety cabinet or super clean platform, slowly add the thawed cell suspension to 5 ml of pre heated Culture Medium for Extracellular Vesicles (EVs).

2.3 After centrifugation at 1000 rpm for 3 min, the supernatant was sucked off and 10 ml of human mesenchymal stem cell culture medium was added to resuspend the cells.

2.4 The cells were evenly spread in the culture dish, and the cells were evenly distributed in the horizontal cross vibration culture dish. The cells were cultured in 37 ℃ constant temperature cell incubator with 5% CO2 for 24 hours, and then the cell status was observed.

2.5 After 24 hours, the Culture Medium for Extracellular Vesicles (EVs) was changed for further culture, and the culture medium was changed every 2-3 days.

Subculture of hMSC cells

1. When the cell fusion degree reaches 90%, the cells can be subcultured.
2. In the super clean table / safe cabinet, suck out the original culture medium, add PBS solution for cleaning once, and add xeno free cell digestive solution (fk-k0204009) to completely cover the bottom of the dish / bottle.
3. Incubate at room temperature for 4-5 minutes or 37 ℃ for 2-4 minutes, and observe under the microscope that most cells will stop digestion when they leave the bottom of the dish.
4. Add the Culture Medium for Extracellular Vesicles (EVs) of twice the volume of digestive fluid, gently blow the cells on the wall of the bottle that are not completely separated with the pipette, and gently blow and mix them to make the cells completely dispersed.
5. The cell suspension was transferred to a 15 ml centrifuge tube and centrifuged at 1000 rpm for 3 min.
6. Discard the supernatant, add the Culture Medium for Extracellular Vesicles (EVs), suspend the cells again, and count. 1:3-1:4, or 1-2x104 cells / cm2, evenly spread in a dish / bottle, and culture in a 37 ℃ 5% CO2 incubator. Note: cell passage time: 2-4 days. The growth rate of 5 generations and earlier cells was faster. After 5 generations, the growth rate of cells was slightly slow.

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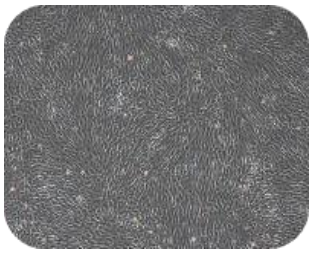
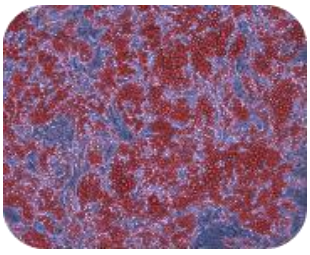
Cryopreservation of hMSC cells

1. The cells reached 90% confluence, the original medium was sucked out and PBS was washed once.
2. Add xeno free cell digestive solution (fk-k0204009) to completely cover the bottom of the dish / bottle, incubate at room temperature for 4-5min or 37 ℃ for 2-4min, and observe under the microscope that most of the cells will stop digestion when they leave the bottom of the dish.
3. Add the Culture Medium for Extracellular Vesicles (EVs) of twice the volume of digestive fluid, blow gently the cells on the bottle wall that are not completely separated with the pipette gun, blow gently and mix well, so that the cells are completely dispersed.
4. The cell suspension was transferred to a 15 ml centrifuge tube and centrifuged at 1000 rpm for 3 min to remove the supernatant.
5. Add proper serum free cell cryopreservation solution (fk-k0204005), adjust the cell cryopreservation density to about 1 × 106 cells / ml, and each cryopreservation tube is separately packed with 1.5-2ml.
6. Put it directly into - 80 ℃ for 24 hours and then transfer it to liquid nitrogen for medium and long-term preservation.

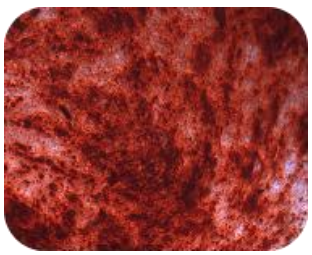
Cell morphogram



Morphology

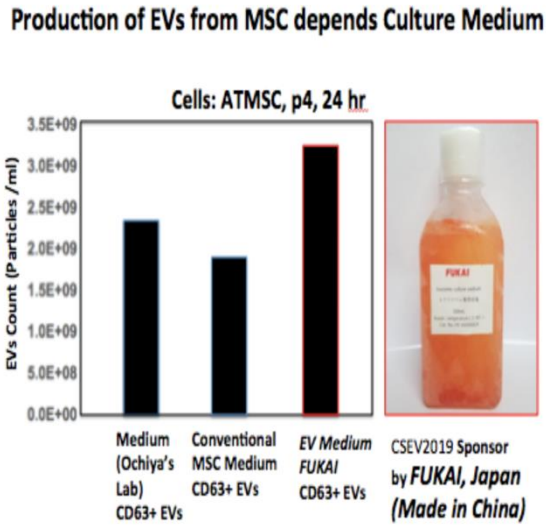


Lipogenesis



Osteogenesis

Cartilage formation



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Quality Control

Test Catagories	Reference Data	The results
Physical APPearance	Orange-red liquid	Conform to the provisions
Clarity	Clear	Conform to the provisions
Ph value	6.9-7.3	Conform to the provisions
Osmolality	270-340 (mosm/KgH20)	Conform to the provisions
Endotoxin	Less than EU/ml	Conform to the provisions
Sterility	Sterility	Conform to the provisions
Mycoplasma	The mycoplasma test was negative after 0.11um filtration	Conform to the provisions
Cell growth test	The cells are spindle - shaped, fingerlike or spiral	Conform to the provisions

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