MSC ACF Tissue Digestive Mix product information

PI-C3501 V1.0

Product Name

Name: MSC ACF Tissue Digestive Mix

Cat. No.: C3501-0020, 3501-0100

Size: 20 mL, 100 mL

Product Description

It is used to digest primary stem cells from tissues and allow subsequent release of mesenchymal stromal cells to migrate from the tissue and proliferate *in vitro* on the substrate. The cultured cells from the digestion are used only for *in vitro* assays and should not be used for therapy.

Isolation of primary mesenchymal stem cells from adult and fetus tissues using specific enzymes is a common cell culture technique. The tissue digestive fluid is prepared using several recombinant digestive enzymes and has no animal-derived components. The tissue digestive fluid is designed for digesting the extracellular matrix of tissues and does not contain any pancreatic enzymes. These enzymes are relatively gentle to the cells and therefore will not harm the digested cells. It can effectively digest the extracellular matrix and isolate a large number of primary mesenchymal stem cells from tissue blocks (umbilical cord, fat, placenta) for subsequent cell proliferation.

Principal Components

Composed of DMEM-LG basal medium, collagenase, dispase, and DNase I.

Application

Primary MSCs from tissues (umbilical cord, adipose, placenta) are harvested for subsequent in vitro proliferation and culture.

Storage and Stability

The product should be kept at **-20°C.**
The product is **light-sensitive** and therefore should not be left in the light.
Shelf life: 12 months from date of manufacture.

Procedure

Taking umbilical cord tissue as an example:

1. Rinse the umbilical cord with DPBS, divide it into 1 – 2 cm segment, and remove blood vessels within the cord.

2. Cut the tissue block into 3 - 5 mm^3 with scissors and knife, transfer them into a 50 mL centrifuge tube, and then add an appropriate amount of enzyme digestive mix solution.

* It is recommended to add 10 mL of enzyme digestive mix solution for an umbilical cord of 10 cm long; the ratio of digestive mix solution (mL): tissue block (g) = 1:1.

** If desired, 1X penicillin-streptomycin (pen/strep) solution may be added to enzyme digestive mix-tissue solution.

3. Place the 50 mL centrifuge tube horizontally in a 37℃ cell culture chamber and incubate for 4 hours to

overnight (16 hours).

* If the total reaction volume is large, it may be mixed by shaking or rotation.

4. Add 0.05% EDTA of the same volume as enzyme digestive mix solution to terminate the reaction, centrifuge at 1,500 x *g* for 5 minutes and remove the supernatant.

* The solution is relatively thick or viscous, and be careful not to disturb the cells layer; it is suggested to leave around 5 mL of solution untouched.

** Without adding EDTA, calcium-, magnesium-free DPBS may be used instead.

5. Resuspend cells with calcium-, magnesium-free DPBS of the same volume as the enzyme digestive mix solution, centrifuge at 500 x *g* for 5 minutes, and remove the supernatant.

6. Resuspend cells with calcium-, magnesium-free DPBS of the same volume as the enzyme digestive mix solution, centrifuge at 250 x *g* for 5 minutes, and remove the supernatant.

7. Resuspend cells with an appropriate amount of culture medium, P0 generation cells may be inoculated for culture using the conventional cell culture methods.

*It is recommended to inoculate over 10,000/cm^2 during the cultivation of primary cells; after passage, it may be cultured according to the conventional inoculation density.

Taking subcutaneous adipose tissue as an example:

1. Rinse fresh subcutaneous adipose tissue blocks with DPBS to remove obvious blood vessels or blood clots.

2. Cut the tissue block into 3-5 mm^3, transfer them into a 50 mL centrifuge tube, and then add an appropriate amount of enzyme digestive mix solution.

*About 5 mL of enzyme digestive mix solution may be added to 1 g of tissue block.

**If necessary, 1X penicillin-streptomycin (pen/strep) solution may be added to enzyme digestive mix-tissue solution.

3. Place the 50 mL centrifuge tube horizontally in a 37℃ incubator and react for 4 hours (the reaction time may be determined by the user).

* If the total reaction volume is large, it may be mixed by shaking or rotation.

4. Add 0.05% EDTA of the same volume as enzyme digestive mix solution to terminate the reaction, centrifuge at 250 x *g* for 5 minutes, and remove the supernatant.

* Without EDTA, calcium-, magnesium-free DPBS may also be used instead.

5. Resuspend cells with calcium-, magnesium-free DPBS of the same volume as enzyme digestive mix solution, centrifuge at 250 x *g* for 5 minutes, and remove the supernatant.

6. Resuspend cells again with calcium-, magnesium-free DPBS of the same volume as enzyme digestive mix solution. After the cell, centrifuge 250 x *g* for 5 minutes and remove the supernatant.

7. Resuspend cells with an appropriate amount of culture medium, filter the cell solution with a 100-mesh sieve, and then inoculate cells on dishes or flasks using conventional cell culture methods.

* After digestion, it is recommended to increase the inoculation density to over 10,000/cm^2 during culture of primary cells; after passage, it may be cultured according to the conventional inoculation density.

Quality Control

MSC ACF Tissue Digestive Mix is tested for sterility, pH, osmolality, and endotoxin concentration. In

addition, each batch is tested for tissue digestion performance.

Note

1. This product is only used for in vitro diagnosis and not for clinical treatment.
2. If the reagent packaging is damaged or leaking, it is strictly prohibited to use.
3. The operation process should avoid contamination.
4. In case of contact with skin and mucous membranes, please rinse with plenty of tap water.
5. Prohibit the use of products that exceed the expiry date.

Manufacturer

Shanghai Dr. Cell Co., Ltd.

Issue Date

June 2023

Precaution and Disclaimer

For research use only, not for clinical diagnosis, and treatment.