Product Name

Name: Tissue Digestive Mix-I

Cat. No.: C3502-0020, C3502-0100

Size: 20 mL, 100 mL

Intended Use

It is used to dissociate primary stem cells from tissues for subsequent isolation and cell culture *in vitro*.

Working principle

Isolation of primary mesenchymal stem cells from tissue blocks using 3 specific enzymes is a common cell culture technique. Tissue Digestive Mix contains several recombinant digestive enzymes and has no animal-derived components. Tissue Digestive Mix is designed for digestion of the extracellular matrix of tissues and does not contain trypsin, which may harm the extracellular receptors on 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 culture.

Main Components

DMEM-LG basal medium, collagenase, dispase, and deoxyribonuclease.

Application

Primary mesenchymal stem cells from tissues (umbilical cord, fat, placenta) can be harvested for subsequent cell culture *in vitro*.

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

Take umbilical cord tissue as an example:

1. Rinse the umbilical cord with DPBS, cut it into 1 - 2 cm long, and remove the blood vessels within the umbilical cord.
2. Cut the tissue block into 3 - 5 mm^3, transfer to a 50 mLcentrifuge tube, and then add an appropriate volume of Tissue Digestive Mix*.
   * For an umbilical cord with a length of 10 cm, it is recommended to add 10 ml Tissue Digestive Mix, or tissue block: Tissue Digestive Mix (vol) = 1:1.
   ** 1% Penicillin-Streptomycin Solution can be added depending on the situation.
3. Put the 50ml centrifuge tube sideways in a 37°C CO_2 incubator for 2 hours (with shaking) to overnight

(16 hours) without shaking.

* The tube can also be continuously rotated and mixed if the total reaction volume is large.

4. Add an equal volume of 0.05% EDTA (as Tissue Digestive Mix) to terminate the reaction, centrifuge at 1500 x *g* for 5 minutes, and remove the supernatant.

   * The solution is relatively thick, so be careful not to affect the underlying cells/tissues during pipetting. It is recommended to leave about 5 ml of liquid.

   ** DPBS (no calcium, no magnesium) can be used instead of EDTA.

5. Resuspend the cells and remaining tissue with the same volume of DPBS, centrifuged at 300 x *g* for 5 minutes, and remove the supernatant.

6. Resuspend the pellet with the same volume of DPBS, centrifuge at 250 x *g* for 5 minutes, and remove the supernatant.

7. Resuspend the pellet with an appropriate volume of culture medium, and P0 cells can be cultured according to the conventional cell culture methods.

   * It is recommended to increase the seeding density of digested primary cells up to 10,000/cm^2 during culture. Conventional seeding density can be used after passage.

Take subcutaneous adipose tissue block as an example:

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

2. Cut the tissue block into 3 - 5 mm^3, transfer to a 50 mL centrifuge tube, and then add an appropriate volume of Tissue Digestive Mix*.

   * For 1 g tissue block, it is recommended to add 10 ml Tissue Digestive Mix, or tissue block: Tissue Digestive Mix (vol) = 1:1.

   ** 1% Penicillin-Streptomycin Solution can be added depending on the situation.

3. Put the 50ml centrifuge tube sideways in a 37°C incubator for 4 hours (the user can also determine the incubation time).

   * The tube can also be continuously rotated and mixed if the total reaction volume is large.

4. Add an equal volume of 0.05% EDTA (as Tissue Digestive Mix) to terminate the reaction, centrifuge at 300 x *g* for 5 minutes, and remove the supernatant.

   * The solution may be relatively thick, so be careful not to affect the underlying cells/tissues during pipetting. It is recommended to leave about 5 ml of liquid.

   ** DPBS (no calcium, no magnesium) can be used instead of EDTA.

5. Resuspend the cells/remaining tissues with the same volume of DPBS, and centrifuged at 250 x *g* for 5 minutes, and remove the supernatant.

6. Resuspend the pellet with the same volume of DPBS, centrifuge at 250 x *g* for 5 minutes, and remove the supernatant.

7. Resuspend the pellet with an appropriate volume of culture medium, filter the cell suspension with a

100-mesh cell strainer, and then inoculate the cells/remaining tissues for culture using conventional methods.

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

Quality Control

Tissue Digestive Mix-I is tested for sterility, pH, osmolality, and endotoxin concentration. In addition, each batch is tested for tissue digestion performance.

Note

1. It is strictly prohibited to take orally or suck liquid through the mouth.
2. If the reagent package is damaged or leaks, it is strictly prohibited to use it.
3. Contamination should be avoided during operation.
4. In case of contact with skin and mucous membranes, please immediately rinse with running water.
5. It is prohibited to use the products that exceed the specified validity period.

Precaution and Disclaimer

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