CiMS™

Instruction for use

CELL SCIENCE & TECHNOLOGY INSTITUTE INC.

ver. 3

Product Description

CiMS™ is a medium for culture of human mesenchymal stem cells (hMSCs). CiMS™ is optimized for the multiple passage expansion of hMSCs (isolated from Bone Marrow, Adipose Tissue and Dental Pulp) while still allowing for differentiation into the several desired lineages. In addition, there is no need to pre-coat culture vessels with any type of attachment matrix before using CiMS™.

Product	Catalog Number (NIPRO/CSTI)	Components	Volume	Storage
CiMS™-BM	87-070 / A2G00P05C	Basal media (Animal-free)	500 mL	2-8 ℃ ; Protect from Light
CiMS™-sAF	87-072 / A2G20P1CC	Animal-free supplement	10 mL	-20 ℃ ; Protect from Light
Related Product	**Catalog Number (NIPRO/CSTI)**	**Components**	**Volume**	**Storage**
rTE	87-974 / 1210	Recombinant Trypsin/EDTA solution	100 mL	2-8 ℃ ; Protect from Light
sTI	87-975 / 1220	Synthetic Trypsin inhibitor	100 mL	2-8 ℃ ; Protect from Light
PBS(-)	87-949, 87-972 / 1102P05, 1102P10	—	500, 1000 mL	1-30 ℃

Storage & thawing of supplement

1. CiMS™ instructions: upon arrival, store CiMS™-BM(basal medium) protected from light at 2℃ to 8℃ and CiMS™-sAF at ≤ -20℃ in a freezer. Quickly thaw a frozen supplement in a 37℃ water bath being careful not to submerge the entire bottle. Watch the supplement closely ; when the whole contents melt, please take it out immediately from the water bath. Once thawed, supplement should be stored 2-8℃ and added to basal medium within 2 weeks. After supplement is added to basal medium, use immediately.

* Fine particles may be observed but there is no influence on quality.

Preparation of Culture Media

1. Decontaminate the external surfaces of the CiMS™-BM and CiMS™–sAF bottle with 70% v/v ethanol or isopropanol.

2. Add 1/50 volume of CiMS™-sAF to CiMS™-BM (CiMS™ complete medium).

* Recommend to make necessary volume of the medium just before use.

Initiation of Culture Process

1. Rapidly thaw a frozen cryotube of hMSC in a 37℃ water bath until a small amount of ice remains.

2. Wipe cryotube with 70% v/v ethanol or isopropanol before opening. Pipet the entire contents of cryotube into a conical tube.

3. Carefully add 10 mL of room temperature (approximately 20-25°C) CiMS™ complete medium.

4. Centrifuge the tubes at about100 x g for 5 minutes at room temperature and discard the supernatant.

5. Resuspend the cell pellet in pre-warm (37℃) CiMS™ complete medium and add the cell suspension to an appropriate culture vessel (cell culture vessels for adherent cells) at a density of 0.5 – 1.0 x 10^4 cells / cm^2.

6. Incubate at 37℃, 5% CO_2, humidified incubator.

Maintenance

1. Change CiMS™ complete medium every 3 days.

Subculturing

The following instructions are for a 25 cm^2 flask. Adjust all volumes accordingly for other size vessels.

1. Subculture the cells when they are about 85% confluent.

2. Remove the culture medium from 25 cm^2 flask.

3. Cover the cells with 0.5 mL rTE (Catalog #87-974) .

4. Place the culture vessel into a 37℃ humidified incubator for 3-5 minutes. Periodically examine the cell layer microscopically and check for cell detachment.

5. Allow the trypsinization to continue until approximately 90% of the cells are rounded up.

6. At this point, tap the flask gently to release the majority of cells from the culture surface. If only a few cells detach, you may not have let them trypsinize long enough.

7. After cell released, neutralize the trypsin in the vessel with 0.5 mL of s-TI (Catalog #87-975) at room temperature.

8. Quickly transfer the detached cells to sterile 15 mL centrifuge tube.

9. Rinse the flask with a final 10 mL of PBS(-)(Catalog #87-949, 87-972) to collect residual cells, and add this rinse to the centrifuge tube.

10. Examine the harvested flask under microscope to make sure the harvest was successful by looking at the number of cells left behind. This should be less than 5%.

11. Centrifuge the tubes at about 100 x g for 5 minutes at room temperature and discard the supernatant.

12. Resuspend the pellet in a minimum volume of temperature equilibrated CiMS™ complete medium by gently pipetting up and down. Count total number of viable cells.

13. Add the calculated volume of cell suspension to each flask and gently rock to disperse the cell suspension over the growth surface.

14. Incubate at 37℃, 5% CO_2, humidified incubator.

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