

CiMS™-U

Instruction for use

ver. 1

Product Description

CiMS™-U/BM is a basal medium especially designed for Human Umbilical Mesenchymal Stromal Cells (HUMSC). Adding the CiMS™-U/sAF to the basal medium results in the complete medium.

Product	Catalog Number (NIPRO/CSTI)	Components	Volume	Storage
CiMS™-U/BM	87-073/A2J10P05C	Serum-free medium for hMSC	500 mL	2-8 °C ; Protect from Light
CiMS™-U/sAF	87-074/A2J20P1CC	Supplement for CiMS™-U/BM	10 mL	below -20 °C ; Protect from Light
Related Product	Catalog Number (NIPRO/CSTI)	Description	Volume	Storage
r-TE	87-974 / 1210	Recombinant Trypsin/EDTA solution	100 mL	2-8 ; Protect from Light
s-TI	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 & stability

Store CiMS™-U/BM (basal medium) at 2°C to 8°C in the dark and CiMS™-U/sAF below -20°C immediately after arrival. Do not freeze the basal medium. If stored properly, the products are stable until the expiry date stated on the label.

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

Prepare complete medium

CiMS™-U/BM requires supplementation with CiMS™-U/sAF.

1. Quickly thaw the frozen supplement in a water bath at 37°C. Once all the contents have thawed, please take it out from the water bath immediately.
2. Aseptically add 1/50 volume of CiMS™-U/sAF to CiMS™-U/BM.
3. Antibiotics can be used if desired, we recommend Gentamicin.

Note

- Recommended concentration of CiMS™-U/sAF is 2%. Optimal concentration should be determined for specific use and cell type.
- Recommend to preparing only the necessary

volume of the medium just before use.

- Do not freeze and thaw CiMS™-U/sAF repeatedly.

Initiation of Culture Process

1. Rapidly thaw a frozen cryotube of hMSC in a 37 °C water bath until a small amount of ice remains.
2. Wipe cryotube with 70% v/v ethanol 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™-U complete medium.
4. Centrifuge the tubes at about 100 x g for 5 minutes at room temperature and discard the supernatant.
5. Resuspend the cell pellet in pre-warm (37 °C) CiMS™-U complete medium and add the cell suspension to an appropriate culture vessel (cell culture vessels for adherent cells) at a density of 0.3 – 1.0 x 10⁴ cells / cm².
6. Incubate at 37 °C, 5% CO₂, humidified incubator.

Maintenance

Change CiMS™-U complete medium every 3 days.

Subculturing

The following instructions are for a 25 cm² flask. Adjust all volumes accordingly for the size of vessels.

1. Subculture the cells when they are about 85% confluent.
2. Remove the culture medium from 25 cm² flask.
3. Cover the cells with 0.5 mL r-TE (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 about 90% of the cells appear rounded.
6. At this point, tap the flask gently to release most of the cells from the culture surface. If only a few cells detach, you may not have allowed them to 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% CO₂, humidified incubator.

Products are for research use only and not intended for human or animal diagnostic or therapeutic uses unless otherwise stated.