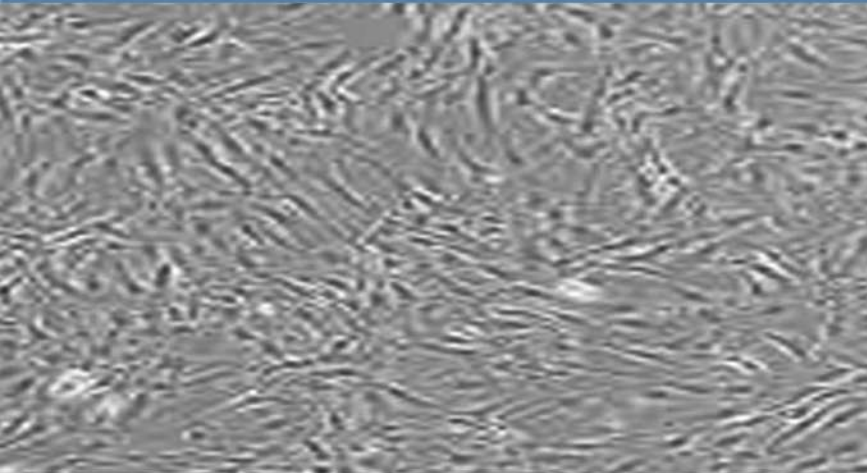


# Human Bone Marrow-Derived Mesenchymal Stem cells (hBM-MSCs)

Cat. No.: 2BM01V



## Intended Use

The normal human Bone Marrow-derived mesenchymal stem cells are an ideal system, may it be drug development studies, any in-vitro research models, organoid cultures, or preclinical studies. Importantly these bone marrow-derived mesenchymal stem cells are primarily used in many therapeutic applications at clinical and pre-clinical levels.

## Product Description

Each vial/flask contains approximately  $1 \times 10^6$  viable cells that are maintained in a suitable medium till the secondary culture stage. The growth supplements and media used for proliferation are selected based on the application of adipose tissue-derived mesenchymal stem cells. While for therapeutic and cosmetic applications, serum-free media is used for optimum growth and maintenance; and for in-vitro research-based applications, any suitable growth medium with a very low percentage of FBS is recommended for optimum growth and proliferation.

In an independent laboratory QC test, the donor blood is collected without anticoagulant after informed consent; and analyzed further for infectious panel markers like HIV, HCV, HBsAg, Syphilis, HPV-1/2, etc. The collected sample is tested for mycoplasma, aerobic/anaerobic bacteria before processing. In our laboratory, each lot of cells is performance tested by culturing cells till passage 2, in a suitable mesenchymal stem cell Growth Medium. During the culture period, no contamination by bacteria, yeast, or fungi was detected. Read the cautious statement carefully. Under optimum cultural conditions, these cells maintain their proliferation capacity and stemness; which is confirmed with the evaluation of expression percentage using specific antibodies, like CD105, CD90, etc. The potency is also confirmed with the assessment of their differentiation potential to adipogenic, chondrogenic, and osteogenic lineages. The cells are maintained at passage 1 and expanded to passage 2 on demand.

Kosheeka ensures complete quality establishments regulations while delivering products, media, supporting reagents, and supplements for optimum performance and reliability.

Contents	Cat. No.	Amount	Storage
Human Bone Marrow-Derived Mesenchymal stem cells (hBMMSCs)	2BM01V	1 Vial/T25 Flask ( $\geq 1 \times 10^6$ cells)	-196°C

**Note:** The Mesenchymal stem cells shall arrive at refrigerated temperature, either in proliferating form in a culture flask or may arrive in dry ice while suspended in a suitable freezing medium. After receipt of the cell vial, immediately analyze it for cell count, viability analysis as well as morphology. Immediately report the same to us. Please be noted that any dissatisfactory information from you after 5 hrs of receipt will not be entertained.

If the cells are not to be used immediately, the cells can be incubated at 37° C till further use. If in case you don't want to use them before 7 days, cells can be detached from the flask after 24 hrs of receipt and cryo-stored with a suitable cryofreeze DMSO medium; with an appropriate storage technique.

In case of the receipt of the vial, it can either be revived with the protocol mentioned herewith or can be stored at -196°c.



## Caution!

Although cells have been tested for the presence of various hazardous agents, diagnostic tests are not necessarily 100% accurate. In addition to the same, human cells and primate cells may harbor other known/unknown pathogens that could be harmful to users.

Kosheeka recommends that appropriate safety procedures be used when handling all primary cells and cell lines, especially those derived from human and primate material. Handle as potential biohazard material using universal precautions.

## The Other Required Material That is not Supplied

Item	Source Recommendation
Human Bone Marrow derived Mesenchymal stem Cell Growth Medium	Any Reputed Manufacturer
Human Mesenchymal stem cells Growth Kit	Any Reputed Manufacturer
Dulbecco's Phosphate Buffer Saline	Any Reputed Manufacturer
Trypsin-EDTA/TrypLE	Any Reputed Manufacturer
Suitable Trypsin-Neutralizer	Any Reputed Manufacturer

## Certificate of Analysis

For Batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.kosheeka.com](http://www.kosheeka.com)

## Growth Conditions

Temperature: 37° C

Atmosphere: 5% CO<sub>2</sub>

## Handling Procedure

### Unpacking and Storage Instruction

- Check all containers for leakage and/or breakage.
- Remove the flask/vial from the packaging and follow the protocol mentioned herewith for the flask as well as the vial.
- Immediately, after the same view the flask under the microscope for any signs of contamination and morphological changes.

## Protocol for Revival of cells in case the cells are shipped in the vial

- Maintain the temperature of 45<sup>0</sup>c in a water bath, and check the same with a suitable lab-grade thermometer.
- Till the time the temperature is achieved, place the vial in the -80 deep freezer only.
- Once the temperature is achieved, take out the vial and rapidly thaw the cells in a water bath by gently swirling them in a clockwise direction.
- Once the media becomes semi-solid, transfer the entire content in 10 ml of complete medium, preconditioned at 37<sup>0</sup>c for 15 mins. Incubate cells in the media for another 10 mins at room temperature.
- Centrifuge at 2000 rpm for 10 mins, with gradual deceleration.

## Protocol for Revival of cells in case the cells are shipped in the flask

- Visualize the flask under the microscope for cell morphology and confluency.
- Replace 10 ml of fresh complete medium and place the flask in the incubator.

## Complete Medium

- **Prepare 1 bottle of mesenchymal stem cells basal medium as follows:**

DMEM/F12 Gibco	500 ml
10% Fetal Bovine Serum	50 ml
L-Glutamine	6mM

\* Along with the same, a combination of antimicrobials is required, which may include Gentamicin-Amphotericin B solution in a concentration of 10 µg/ml and 0.25 µg/ml.

- **Counting the total number of viable cells, initial seeding density can be defined. Recommended are 5000 cells per cm<sup>2</sup>.**
- **Prepare the desired quantity of flasks/plates by adding approximately 7 ml of growth medium per 25cm<sup>2</sup> of surface area. Place the flasks/plates in incubators at 37<sup>0</sup> C with 5% CO<sub>2</sub>**

## Subculturing procedures

- Passage of normal human adipose-derived mesenchymal stem cells when the flask has reached 70-80% confluency and is actively proliferating.
- Warm TrypLe-for primary cells at 37° C, before use along with complete growth medium.
- For each flask, carefully aspirate the spent medium without disturbing the monolayer.
- Briefly rinse the cell layer with 3-5 ml of DPBS to remove residual traces of media and then discard the DPBS.
- Add pre-warmed trypsin EDTA solution of approximately 3 ml to each flask.
- Gently rock each flask and keep the same in the incubator to ensure optimum temperature incubation.
- After 3 mins, take out the flask, and observe the cells under the microscope. When cells pull away from each other and round up, flasks can be tapped gently from several sides to promote detachment.
- Upon detaching the majority of cells, quickly add a suitable neutralizing solution to neutralize trypsin. Gently swirl the culture and centrifuge to get the pellet.
- Centrifugation should be carried out at 2000 rpm for 10 mins, count the cells, and seed new flasks at recommended density.
- Place freshly seeded flasks in the incubator and maintain them with suitable media.

## Troubleshooting

Observation	Portable Cause	Recommended Solution
Damage to cultured BMMSCs	Damage can occur during trypsinization. This may be due to longer exposure to Trypsin/TrypLE. The damage can also be about an improper neutralization process.	Ensure the appropriate temperature of trypsinization.
	Damage can also occur due to inappropriate centrifugation at a higher speed.	Ensure the speed of centrifugation is appropriate.
	Cells can be damaged due to higher seeding density during passage and keeping them in culture for a longer time.	Ensure optimized seeding density for appropriate confluence.

## Product Citation

If the use of this product results in a scientific publication, please cite the product in the following manner: Primary Human Bone Marrow-Derived Mesenchymal Stem cells (Kosheeka: 2BM01V).

## References

References and other information on this product are available at [www.kosheeka.com](http://www.kosheeka.com)

## Warranty

The product provided by Kosheeka is warranted for viability for 24 hrs from the date of shipment, provided that the customer has stored and handled the product according to the information, included on the product information sheet, website, and certification of analysis. For living cultures, Kosheeka lists the media formulation and reagents that are effective for the product and hence, recommended. However, products from any reputed manufacturer can be used. A change in the protocol may affect the outcome in terms of growth, viability, and/or functional characteristics of cells. However, for alternative use of media, Kosheeka is not liable for any discrepancy.

## Disclaimer

The product is intended for laboratory research use only. It is not intended for any human/animal therapeutic purpose.

The product is sent on the condition that the customer is responsible and knowledgeable for handling and assumes all risk and responsibility in connection with the receipt, handling, storage, disposal, and use of the Kosheeka product including without limitation taking all appropriate safety and handling precautions to minimize health or environmental risk.