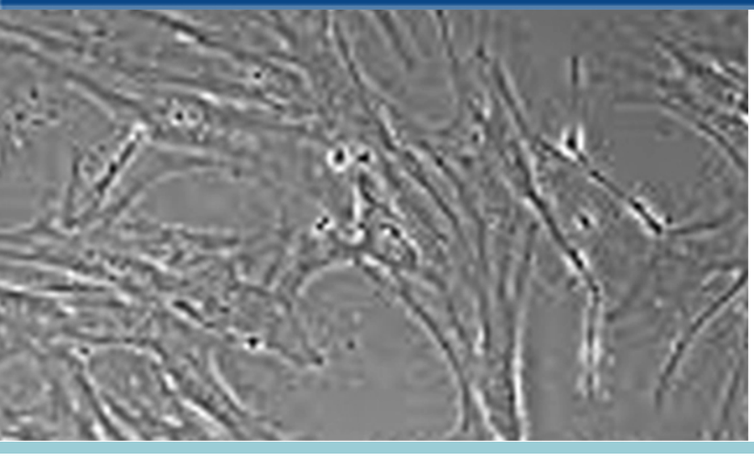
Human Adipose Tissue-Derived Stromal Vascular Fraction Cells

Cat. No.: hAT-1102



Intended Use

The human adipose tissue-derived stromal vascular fraction contains a heterogenous population of a number of potent cells, including mesenchymal stem cells, connective precursors, preadipocytes, endothelial precursors, regulatory T cells, macrophages, smooth muscle cells, pericytes, etc. The population of cells is primarily used for a number of applications, including cosmetic applications, therapeutic benefits for joint disorders, wound healing, and in-vitro research purposes. These cells are also used as ideal models for the number of clinical implications like diabetes, obesity, etc.

Product Description

Each vial/flask contains approximately 1 x 10^7 viable cells that are maintained in a suitable medium as a non-adherent population. The cells are isolated from the outer stroma of visceral fat using a suitable enzyme like collagenase, at an optimum concentration of 0.1 mg/ml. The cells if intended to use for therapeutic applications are treated using GMP-grade reagents, enzymes, and products; without affecting their outcome and potency.

The cells are maintained in a non-adherence state in culture using a suitable growth medium for optimum proliferation. The details of the same are provided in subsequent sections.

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, CMV, etc. The collected sample is tested for mycoplasma, aerobic/anaerobic bacteria before processing. In our laboratory, each lot of cells is performance tested for cell growth, viability, and periodical contamination, such as bacteria, yeast, and/or fungi.

Under optimum cultural conditions, these cells maintain their proliferation capacity and stemness; which is confirmed with the evaluation of expression percentage using specific anti-bodies, like CD 31, CD44, etc.

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 Adipose Tissue-Derived Stromal Vascular Fraction (hADSVFs)	hAT-1102	1 Vial (≥ 1 x 10 7 cells)	Refrigerated Temperature

Note: The Stromal Vascular Fraction shall arrive at refrigerated temperature, while suspended in platelet lysate. After receipt of the cell vial, immediately analyze it for cell count and viability analysis. 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 stored with a suitable cryofreeze DMSO medium; with an appropriate storage technique.

⚠ 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 Fibroblast Basal Medium	Any Reputed Manufacturer
Human Fibroblast derived Growth Kit	Any Reputed Manufacturer
Dulbecco's Phosphate Buffer Saline	Any Reputed Manufacturer
Culture vessels like roller bottles, biofactories, etc.	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

Growth Conditions

Temperature: 37^0 c
Atmosphere: 5% CO_2
Continuous shaking

Handling Procedure

Unpacking and Storage Instruction

- Check all containers for leakage and/or breakage.
- Remove the vial from the packaging and keep it in an incubator for around 15-20 mins, for stabilizing cells.
- Immediately, after the same pull the volume with phosphate buffer saline; and centrifuge to pellet the cells.
- Check for the cell count and viability of the product immediately.

Complete Medium

- **Prepare 1 bottle of fibroblast basal medium as follows:**

  500 ml Gibco Serum Free Fibroblast Basal Medium
  Other media supplements like a recommended dose of Gentamicin-Amphotericin B (Recommended Dose: Gentamicin- 10 µg/ml and Amphotericin B- 0.25 µg/ml)

- **Add the recommended quantity of the below-mentioned chemicals for optimum growth and development of vaginal epithelial cells.**

Components	Final Concentration
rhFGF Beta	0.5 ml, 5 ng/ml
L-glutamine	18.75 ml, 7.5mM
Ascorbic Acid	0.5 ml, 50 µg/ml
Hydrocortisone hemisuccinate	0.5 ml, 1 µg/ml
rhinsulin	0.5 ml, 5 µg/ml
Fetal Bovine Serum (Optional)	2%

- **Counting the total number of viable cells, initial seeding density can be defined. Recommended are 5000 cells per cm2.**
- **Prepare the desired quantity of culture vessels by adding approximately 7 ml of growth medium per 25cm2 of surface area. Place the flasks/plates in incubators at 37^0c with 5% CO_2 .**
- **Place the culture flasks on shaking incubators.**
- **Let the cells proliferate till desired cell number is achieved.**

Subculturing procedures

- While desired cell count is achieved, collect all the media along with suspended cells in 50ml centrifuge tubes.
- Centrifuge all the tubes at 2000 rpm for approximately 15 mins at zero deceleration, in order to pellet down the cells.
- Discard the supernatant medium and replace the same with 5 ml fresh growth medium, in each tube.
- Divide the content of the tube into two parts, and transfer the same to other fresh culture vessels.
- Top up with the required amount of growth medium.

Cryopreservation

- Collect the entire content of each flask to be cryopreserved in a 50 ml centrifuge tube.
- Centrifuge at 2000 rpm for approximately 10 mins.
- Discard the supernatant, and replace the same with a suitable cryopreservation medium along with DMSO. Approximately 10 x 10 6 cells can be stored in 5 ml of cryopreservation media.
- With the help of a controlled rate freezer, freeze the cells till required usage.

Troubleshooting

Observation	Portable Cause	Recommended Solution
Damage to stromal vascular fraction cells	Damage can occur during trypsinization. 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.

Product Citation

If the use of this product results in a scientific publication, please cite the product in the following manner: Primary Human Adipose Tissue-Derived Mesenchymal Stem cells
(Kosheeka: **hAT-1102**)

References

References and other information on this product are available at 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.