

Human Epidermal Melanocytes, adult (HEMa-LP)

Cat. no. C-024-5C

Quantity: $\geq 500,000$ viable cells/vial

Product Description

HEMa-LP are human epidermal melanocytes isolated from **lightly pigmented** (LP) adult skin. Each vial of this product contains $\geq 5 \times 10^5$ viable cells that have been cryopreserved at the end of the secondary culture stage in a medium containing dimethylsulfoxide (DMSO). The cells in each lot stain positively with Mel-5 antibody^{1,2} in the fourth culture after thawing. An independent laboratory tests the cells for the presence of mycoplasma, Hepatitis B, Hepatitis C, and HIV-1 viruses. These agents were not detected. In our laboratory, each lot of cells is performance tested by culturing the cells through multiple passages in Medium 254 supplemented with Human Melanocyte Growth Supplement-2 (HMGS-2, Cat. no. S-016-5) in the absence of antibiotics and antimycotics. During this culture period, no contamination by bacteria, yeast, or fungi was detected. Upon thawing, the cells are guaranteed to be $\geq 70\%$ viable and to have a potential of ≥ 16 population doublings when handled according to the directions provided in this document. For recommended precautions to be taken when handling human cells, please read the caution statement.

Intended Use

Cryopreserved HEMa-LP are intended for use by researchers investigating the molecular and biochemical basis of various normal and disease processes. **This product is for research use only. Not for use in animals, humans, or diagnostic procedures.**

Storage and Stability

Cryopreserved HEMa-LP should arrive frozen on dry ice. If the cells are not to be used immediately, prepare a space for storage of the vial in the vapor phase of a liquid nitrogen freezer. While wearing protective eyewear, gloves, and a laboratory coat, remove the vial from its shipping container and place immediately in the liquid nitrogen freezer. Although the viability of cryopreserved cells decreases with time in storage, useful cultures can usually be established even after 2 years of storage at liquid nitrogen temperatures.

Caution

Although cryopreserved cells have been tested for the presence of various hazardous agents, diagnostic tests are not necessarily 100% accurate. In addition, human cells may harbor other known or unknown agents, or organisms which could be harmful to your health or cause fatal illness. Treat all human cells as potential pathogens. Wear protective clothing and eyewear. Practice appropriate disposal techniques for potentially pathogenic or biohazardous materials. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Initiating Cultures from Cryopreserved Cells

We recommend seeding cells recovered from cryopreservation at a density of 5×10^3 viable cells/cm². For example, four 25 cm² tissue culture flasks can usually be established from one vial containing $\geq 5 \times 10^5$ HEMa-LP. The procedure given below is a sample protocol for establishing cultures from the contents of one vial.

1. Prepare a bottle of supplemented Medium 254 (Cat. no. M-254-500) according to the instructions supplied with that product.

Note: Although Medium 254 may be supplemented with PMA-Free Human Melanocyte Growth Supplement-2 (HMGS-2, Cat. no. S-016-5) or with traditional Human Melanocyte Growth Supplement (HMGS, Cat. no. S-002-5), we recommend the use of PMA-Free Human Melanocyte Growth Supplement-2 (HMGS-2) for optimal performance.

2. Warm the supplemented culture medium to 37°C in a water bath.
3. Remove a vial of HEMa-LP from liquid nitrogen storage, taking care to protect hands and eyes.
4. Dip the lower half of the vial into a 37°C water bath to thaw.
5. When the contents of the vial have thawed, wipe the outside of the vial with disinfecting solution and move to a Class II, type A laminar flow culture hood.
6. Open the vial and pipet the suspension up and down with a 1 ml pipette to disperse the cells.
7. Remove 20 μ l from the vial and dilute the cell suspension in 20 μ l of trypan blue solution (Cat. no. 15250-061).
8. Using a hemacytometer, determine the number of viable cells per ml.
9. Dilute the contents of the vial (1 ml) to a concentration of 2.5×10^4 viable cells/ml using the supplemented medium from step 1, above.
10. Add 5 ml of cell suspension to each 25 cm² culture flask.
11. Following inoculation, swirl the medium in the flasks to distribute the cells.
12. Incubate the cultures in a 37°C, 5% CO₂/95% air, humidified cell culture incubator. For best results, do not disturb the culture for at least 24 hours after the culture has been initiated.

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Maintaining Stock Cultures

1. Change the culture medium to freshly supplemented Medium 254 24 to 36 hours after establishing a culture from cryopreserved HEMa-LP. For subsequent subcultures, change the medium 48 hours after establishing the subculture. For best results and highest growth rates, warm the medium to 37°C before use.
2. Change the medium every other day thereafter until the culture is approximately 80-90% confluent.
3. Once the culture reaches 80-90% confluency, change the medium every day.

Notes

- To achieve the highest cell densities, change the culture medium every day as the cultures approach confluence. The number of subcultures (passages) that can be achieved will vary with the starting cell density and the methods employed by individual investigators. HEMa-LP cultures grow slowly.
- HEMa-LP cultures seeded at 5.0×10^3 cells/cm² from cryopreserved cells should reach 80% confluency in 12-16 days. In this culture, most of the cells should have a multipolar morphology. Occasionally, small numbers of keratinocytes persist in the tertiary culture. In older cultures, more cells may become bipolar in appearance.

Subculturing HEMa-LP

View the culture under a microscope to ascertain the condition of the culture (i.e., confluence, mitotic activity). This protocol is designed for the subculture of one 25 cm² culture flask. If different-sized culture vessels are to be used, reagent volumes should be adjusted accordingly.

1. Assemble subculture reagents and materials:
 - Medium 254 supplemented with HMGS-2 or HMGS

Note: For optimal performance, we recommend the use of PMA-Free HMGS-2 (Cat. no. S-016-5).

- Trypsin/EDTA solution (Cat. no. R-001-100)
- Trypsin Neutralizer solution (Cat. no. R-002-100)
- Culture vessels (not provided)
- Sterile pipettes (not provided)
- Sterile 15 ml conical tubes (not provided)

Note: Do not warm the supplemented medium or other reagents to 37°C prior to use.

2. Remove all of the culture medium from the flask.
3. Add 4 ml of Trypsin/EDTA solution to the flask. Rock the flask to ensure that the entire surface is covered.
4. Immediately remove 3 ml of Trypsin/EDTA solution from the flask.

5. Incubate the flask at room temperature for approximately 1-3 minutes.
6. View the culture under a microscope. Continue the incubation until the cells have become nearly completely round with a few small processes remaining.
7. Tap the flask gently to dislodge cells from the surface of the flask.
8. Add 3 ml of Trypsin Neutralizer solution to the flask and transfer the detached cells to a sterile 15 ml conical tube.
9. Add 3 ml additional Trypsin Neutralizer solution to the flask and pipette the solution over the flask surface several times to remove any remaining cells. Add this solution to the 15 ml conical tube.
10. Centrifuge the cells at $180 \times g$ for 7 minutes. Observe the cell pellet.
11. Remove the supernatant from the tube, being careful not to dislodge the cell pellet.
12. Resuspend the cell pellet in 4 ml supplemented Medium 254. Pipet the cells up and down with a 10 ml pipette to ensure a homogeneous cell suspension.
13. Determine the concentration of cells in the suspension.
14. Dilute the cells in supplemented Medium 254 and seed new culture vessels with 5×10^3 cells/cm².
15. Incubate the cultures in a 37°C, 5% CO₂/95% air, humidified cell culture incubator.

Notes

- Damage to cultured HEMa-LP can occur during trypsinization. This damage may result from exposure of the cells to the Trypsin/EDTA solution for excessive lengths of time, trypsinization at temperatures exceeding room temperature and/or excessive mechanical agitation.
- Another common source of damage is centrifugation at excessive g forces. Check to make sure that the speed of the centrifuge is appropriate. One manifestation of cellular damage that may be evident after centrifugation is strings of cells (and debris) that do not pellet in the bottom of the tube. This is due to the presence of DNA from lysed cells in the solution. If this condition exists, the cell pellet may be lost upon aspiration of the supernatant containing the DNA strings. In many cases, viable cells can be rescued by pipetting the cells (and DNA) up and down in a 10 ml pipette to shear the DNA, and centrifuging the suspension again to recover the cells.

Selected References

1. Bhawan, J., *Melanoma Res.* 7(1): 43-8, 1997.
2. Swope, V.B., Supp, A.P., and Boyce, S.T., *Wound Repair Regen.* 10(6): 378-86, 2002.

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