



HEScGRO™ ANIMAL-COMPONENT-FREE MEDIUM FOR CULTURE OF HUMAN EMBRYONIC STEM CELLS

CATALOG NUMBER: SCM020

LOT NUMBER:

QUANTITY: 500 mL of HEScGRO™ Serum-Free Medium for hES cells

DESCRIPTION: HEScGRO™ hES Medium is designed to support the growth and expansion of undifferentiated human ES cells, and is specially formulated to meet the special requirements of human embryonic stem cell culture. It has been successfully tested and proven to maintain the pluripotential nature of the following hES cells: MEL-1, MEL-2, MEL-4, H1 and H9. The media is defined, serum-free, and complete in a “ready to use” format to maintain cells in their pluripotent state. Mitotically inactive human feeder cell layers are required to support hES cell growth. **PLEASE NOTE: Human feeder cells are required for successful hES cell culture. Mouse feeder cells are not recommended with this medium.**

COMPOSITION: HEScGRO™ Medium is a proprietary, patent-pending formulation. The medium formulation does not contain any animal derived components. It comes as a complete “ready to use” formulation with (20 ng/mL) bFGF included.

QUALITY CONTROL: Each lot of medium is tested for the optimal cell growth and proliferation of H9 Human ES cells
Sterility Testing: Negative
Osmolarity: 260-270 mOsm
pH: 7.2-7.3

MATERIAL REQUIRED BUT NOT SUPPLIED:

- Tissue Culture plates
- Matrigel, 0.1 % gelatin solution, human collagen IV or other tissue culture plastic coating material.
- Mitotically-inactivated Detroit 551 (ATCC cat no. CCL-110) or WS1 (ATCC cat no. CRL-1502) human fibroblast feeder cells are recommended, plated at 60,000 cells/cm².
- **IMPORTANT: Human feeder cells are required for successful hES cell culture. Mouse feeder cells are not recommended with this medium.**

STORAGE AND HANDLING:

HEScGRO™ Serum-Free Medium should be stored at -20°C until ready to use. Upon thawing, the medium should be stored at 2-8°C and given a 2 week expiration dating. Dispense into aliquots to avoid repeated heating prior to each use.

PROTOCOLS:

Specific culture protocol for human ES cells will vary widely depending on the cell type, and may require optimization for best results. The following protocols are generic guidelines.

Manual Passaging of Human ES cells in HEScGRO

- 1) Coat culture vessel with appropriate substrate. Examples of commonly used substrates for human ES cell culture are 0.1% gelatin, Matrigel, or collagen. Apply substrate as for standard human ES cell culture.
- 2) At least one day prior to plating the human ES cells, plate feeder cells onto the coated culture surface using standard fibroblast media (e.g., 90% DMEM (with 4 mM L-glutamine, 1.5 g/L sodium bicarbonate and 4.5 g/L glucose), 10% fetal bovine serum). Human feeder cells are required for culture with Millipore's HEScGRO™ serum-free media. Typically, a human fibroblast feeder line such as Detroit 551 are plated at a density of 60,000 cells per cm² (feeder cells should be inactivated by either Mitomycin C treatment or irradiation prior to use).
- 3) Human ES cells are normally passaged manually when using Millipore's HEScGRO™ serum-free media. To passage human ES cells manually, first replace the media in the culture with fresh, pre-warmed HEScGRO™ serum-free media. Next, divide a single colony into small pieces (using a pulled glass capillary or a 10 µL pipet tip), and lift the pieces from the culture surface. When all colonies to be passaged have been divided and lifted, gently remove all of the culture media (containing the lifted pieces) and transfer to the culture vessel containing the feeder cells plated the day before (rinse the feeders once with D-PBS before adding the human ES cells, or feeders can be serum-starved prior to adding human ES cells). Add additional media as necessary and return the culture vessel to the 37°C incubator. It is also possible to do "bulk" passaging by other methods, for example using dissociation reagents such as Accumax™ (Catalog No. SCR006).
- 4) The HEScGRO™ medium in human ES cell cultures should be replaced daily. Pre-warm the media to 37°C before adding to human ES cell cultures.
- 5) After five to seven days, the human ES cells should be ready for passaging.

Passaging of Human ES Cells in HEScGRO with Accumax

- 1) Prepare plated human fibroblast feeder cells as in steps 1 and 2 above.
- 2) Remove HEScGRO culture medium from the culture vessel, and replace with 0.1 ml of Accumax per square centimeter of culture surface. Incubate vessel at 37°C for exactly five minutes (it is important that the dissociation with Accumax not go longer than five minutes). Quench the dissociation by adding 1 ml HEScGRO (prewarmed to 37°C) for each ml of Accumax used, and scrape the culture surface with a 5 ml serological pipet. Remove the dissociated cells (cells will actually be in small clumps, not as individual cells) to a 15 ml conical tube, and wash the culture surface with additional HEScGRO; add this to the conical tube and centrifuge cells for 5 minutes at 75g. After centrifugation is complete, aspirate and wash cells with several ml of HEScGRO and centrifuge again. After aspiration, resuspend cells in fresh HEScGRO and add cells to the pre-plated human fibroblast cultures at a split ratio from 1:3 to 1:10, depending on the density of the starting culture (note: wash the pre-plated fibroblasts once with sterile Dulbecco's PBS before adding the human ES cells in HEScGRO).
- 3) Feed the human ES cells with fresh HEScGRO medium daily. Pre-warm the media to 37°C before adding to human ES cell cultures.
- 4) After five to seven days, the human ES cells should be ready for passaging.



Passaging of Human ES Cells in HEScGRO with Collagenase type I

- 1) Prepare plated human fibroblast feeder cells as in steps 1 and 2 above.
- 2) Remove HEScGRO culture medium from the culture vessel, and replace with 0.1 ml of 4 mg/ml collagenase type I solution per square centimeter of culture surface. Incubate vessel at 37°C for 5 minutes, and then check for curling at the edges of colonies. If little or no curling is observed, put vessel back at 37°C for an additional 5 minutes, and repeat until curling is observed. When the cells are ready, wash cells off of the culture surface with 3 ml of HEScGRO medium per ml of collagenase type I solution (note: do NOT scrape the cells from the culture surface). Collect the cells in a 15 ml conical tube, and centrifuge at 75g for 5 minutes. After the centrifugation is complete, aspirate and wash cells with fresh HEScGRO medium before repeating centrifugation. After aspiration, resuspend cells in fresh HEScGRO and add cells to the pre-plated human fibroblast cultures at a split ratio from 1:3 to 1:6, depending on the density of the starting culture (note: wash the pre-plated fibroblasts once with sterile Dulbecco's PBS before adding the human ES cells in HEScGRO).
- 3) Feed the human ES cells with fresh HEScGRO medium daily. Pre-warm the media to 37°C before adding to human ES cell cultures.
- 4) After five to seven days, the human ES cells should be ready for passaging.

GENERAL REFERENCES:

1. Hyslop LA *et al.* (2005). *Expert Reviews in Molecular Medicine* **7**(19):1-21.
2. Wobus AM and Boheler KR. (2005). *Physiological Rev* **85**:635-678.
3. Hoffman LM and Carpenter MK.(2005). *Nature Biotech* **23**(6):699-708.
4. Schatten G *et al.* (2005). *Nature Methods* **2**(6):455-463.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 μ L or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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