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HEScGRO™Basal Medium
Animal-Component-Free Medium
for hES Cell Culture Applications

CATALOG NUMBER: SCM021

LOT NUMBER:

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

DESCRIPTION: HEScGRO™ Basal hES Medium is a basal formulation (without growth factors) designed to support the growth and expansion human ES cells, and is specially formulated to meet the special requirements of human embryonic stem cell culture. It. The media is defined, serum-free, and animal component-free. **PLEASE NOTE: Human feeder cells are required for successful hES cell culture. Mouse feeder cells are not recommended with this medium.**

COMPOSITION: HEScGRO™ Basal Medium is a proprietary, patent-pending formulation. The medium formulation does not contain any animal derived components. The Basal Formulation does not have growth factors added, and will not maintain undifferentiated expansion of hES cells without the addition of growth factors. (For expansion of undifferentiated hES cells, please use SCM020, HEScGRO Medium, which contains 20ng/ml of bFGF)

QUALITY CONTROL: 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 feeder cells are recommended (ATCC cat no. CCL-110) plated at 80,000 cells/cm^2 .
- Other feeder cells such as HS27 or WS1 cells may also be used, typically plated at 55,000 cells/cm^2.
- **IMPORTANT: Human feeder cells are required for successful hES cell culture. Mouse feeder cells are not recommended with this medium.**

STORAGE AND HANDLING:

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

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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 protocol is a generic guideline.

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). Feeder cells are required for culture with Chemicon's HEScGRO™ serum-free media. Typically, a human fibroblast feeder line such as Detroit 551 are plated at a density of 80,000 cells per cm^2 (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 Chemicon'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 37ºC incubator. It is also possible to do "bulk" passaging by other methods, for example using dissociation reagents such as Accutase™ (Catalog No. SCR005).

4) The HEScGRO™ medium in human ES cell cultures should be replaced daily. Pre-warm the media to 37ºC before adding to human ES cell cultures.

5) 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.

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