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Human Embryonic Stem (ES) Cell Embryoid Body Formation Medium

CATALOG NUMBER: SCM026

LOT NUMBER:

QUANTITY: 5 x 100 mL bottles

DESCRIPTION: Human Embryonic Stem (ES) Cell Embryoid Body Formation Medium is a formulation that is designed for the spontaneous or directed differentiation of human embryonic stem cells.

Embryonic stem cells are pluripotent cells derived from the inner cell mass of preimplantation embryos and have the ability to differentiate into cells comprising all three embryonic germ layers. One method to identify the pluripotent potential of hES cells is the induction of *in vitro* differentiation through the culture of human ES cells in suspension to form embryoid bodies. Embryoid bodies are subsequently evaluated for the presence of markers for ectoderm, mesoderm, and endoderm. Additionally, embryoid bodies are considered to provide an environment that mimics early embryonic development and can hence be used for the differentiation of hES cells towards specific lineages.

Millipore's Human ES Cell Embryoid Body Formation Medium has been optimized and qualified to support the formation of embryoid bodies. The medium can be used to form embryoid bodies in suspension culture on low adhesion plates. Embryoid bodies formed using SCM026 have been shown to facilitate the differentiation of human ES cells into neural, endodermal, and cardiac cell lineages.

PRESENTATION: Human ES Cell Embryoid Body Formation Medium is a proprietary formulation that is based on published human ES cell embryoid body media (1-4) and contains 20% fetal bovine serum (ES Qualified), glutamine, nonessential amino acids, and β-mercaptoethanol.

QUALITY CONTROL: Each lot of medium is tested for the ability to support human ES cell embryoid body formation. Sterility Testing: Negative.

MATERIALS REQUIRED/RECOMMENDED BUT NOT SUPPLIED:

- Cryopreserved Human Embryonic Stem Cells (Catalog Nos. SCC020 and SCC021).
- HEScGRO Medium™ for Human ES Cell Culture (Catalog No. SCM020).
- Accumax™ (Catalog No. SCR006).
- Dulbecco's Phosphate-Buffered Saline (1X PBS) (Catalog No. BSS-1005-B).
- 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 60,000 cells/cm^2 .
- Low attachment plates.

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STORAGE AND HANDLING:

Human Embryonic Stem Cell Embryoid Body Formation 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 3 week expiration dating. Dispense into aliquots to avoid repeated heating prior to each use. The media can also be dispensed into aliquots and refrozen at -20°C although repeat freeze-thaws are not recommended.

PROTOCOLS:

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

Formation of Embryoid Bodies:

*Note: Human ES cells should be of high quality. Undifferentiated ES cells are characterized by distinct colonies with a high nucleus to cytoplasm ratio, high alkaline phosphatase levels, and distinct phase bright borders. It is recommended that embryoid bodies be formed with cells that are at a density that requires passaging. The protocol below is for human ES cells grown in HEScGRO™ media. Millipore's Human ES Cell Embryoid Body Formation Medium has also been used successfully on human ES cells grown in Knockout Serum Replacement conditions (**Figure 1B**). In Knockout Serum Replacement conditions, cells can either be scraped directly from the plate to make embryoid bodies or passaged using collagenase IV or other enzymatic methods.*

1. Using a 5 mL serological pipette, gently scrape cells off dish. To ensure uniformity in size, a criss-cross, grid-like pattern can be made on the dish with the serological pipette. Using the serological pipette, gently pipette up and down to dislodge the cells. Additional scraping may be required.
2. Rinse the well with Human ES Cell Embryoid Body Formation Medium to remove all additional cells.
3. Allow cells to sediment to the bottom of a 15 mL conical tube through gravity pelleting (approximately 10 minutes).
4. Aspirate the supernatant being careful not to disturb the pellet. It is recommended to leave an additional 3 mL of media remaining so as not to disrupt the pellet.
5. Rinse the pellet once with 10 mL Human ES Cell Embryoid Body Formation Medium to wash away any remaining HEScGRO™ Medium**.**
6. Resuspend pellet gently in 4-5 mL of Human ES Cell Embryoid Body Formation Medium and transfer cells to one well of a 6-well low adhesion plate. A split ratio of 1:1 to 2:1 (one well of 6-well plate of human ES cells into one well of 6-well low adhesion plate) is recommended but may differ depending on starting cell densities.
7. Incubate cells for desired number of days in suspension changing media every 2-3 days by allowing the embryoid bodies to settle by gravity and resuspending embryoid bodies in fresh Human ES Cell Embryoid Body Formation Medium (**Figure 1A**).
8. The embryoid bodies can be plated on gelatin-coated or other adherent dishes at different stages for further differentiation. Differentiated cells can be analyzed by immunocytochemistry and/or RT-PCR to detect lineage specific markers.

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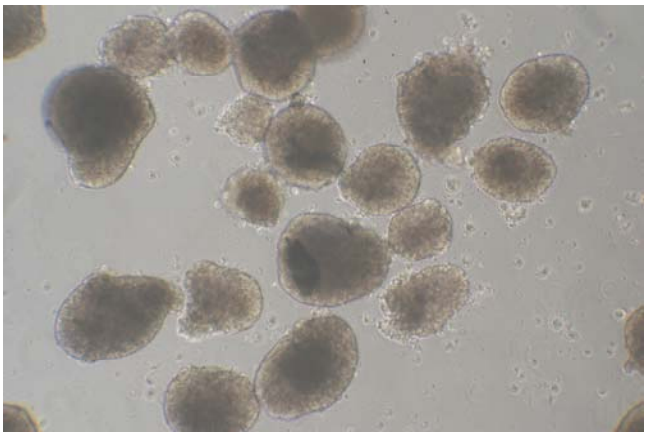
A.



B.

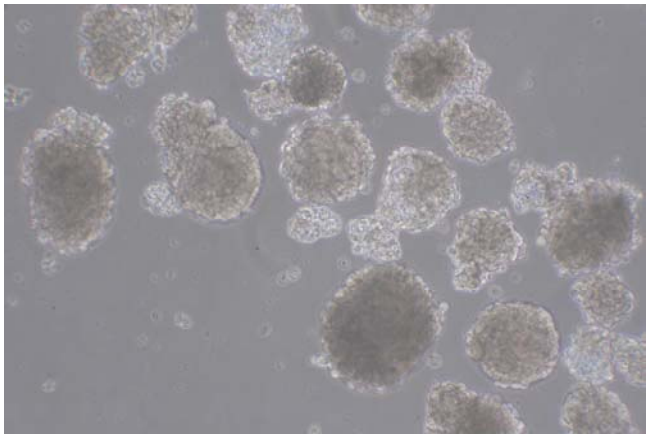


Figure 1. Representative images of EBs formed by the culture of human ES cells (Catalog No. SCM026) in human ES Cell Embryoid Body Formation Medium. **(A)** Day 7 embryoid bodies formed from H9 human ES cells cultured for ten passages in HEScGRO™ media. **(B)** Day 2 embryoid bodies formed from H9 human ES cells cultured in Knockout Serum Replacement media conditions.

GENERAL REFERENCES:

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4. Takahashi K. *et al.* (2007). **Cell** 131:1-12.
5. Keller G. (2005). *Genes Dev* **19**: 1129-1155.
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7. Trounson A. (2006). *Endocrine Reviews* **27**:208-219.

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