upstate | CHEMICON | Linco
THE EXPERTISE OF UPSTATE®, CHEMICON® AND LINCO® IS NOW A PART OF MILLIPORE

EMBRYOID BODY (EB) FORMATION MEDIUM

CATALOG NUMBER: SCM018

LOT NUMBER:

QUANTITY: 100 mL

BACKGROUND:

Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass (ICM) of preimplantation embryos and have the unique ability to differentiate into cells comprising all three embryonic germ layers (ectoderm, mesoderm and endoderm). Most protocols used to differentiate murine ES cells to a variety of cells involve an initial step in which the spontaneous differentiation of ES cells proceed through the formation of embryoid body (EB). EBs are formed by culturing ES cells in suspension culture in low attachment Petri dishes. The non-adhesive surfaces of Petri dishes promote the aggregation of ES cells into a tight ball of cells termed embryoid body. EBs are thought to help maintain an embryonic organization and closely mimic some of the earliest stages of embryonic development.

Millipore's Embryoid Body (EB) Formation Medium has been optimized and qualified to support the formation of embryoid body. The medium can be used to form EB from hanging drops or suspension culture on non-adhesive Petri dishes. EB's formed using SCM018 have been shown to aid in the differentiation of mouse ES cells into neural, adipogenic and cardiomyogenic cell lineages.

PRESENTATION:

Embryoid Body (EB) Formation Medium is a proprietary formulation that contains fetal bovine serum. Sterility Testing: Negative

MATERIALS REQUIRED BUT NOT SUPPLIED:

- Cryopreserved Mouse Embryonic Stem Cells (Catalog Nos. SCR011, SCR012, SCC013, CMTI-1 and CMTI-2)
- EmbryoMax® Complete ES Cell Media w/15% FBS and LIF (Catalog No. ES-101-B)
- ESGRO® Mouse LIF Medium Supplement (Catalog No. ESG1107)
- Accutase™ Cell Dissociation Solution (Catalog No. SCR005)
- Phosphate-Buffered Saline (1X PBS) (Catalog No. BSS-1005-B)
- Bacteriological Petri dishes or ultra low attachment Petri dishes

28820 Single Oak Drive • Temecula, CA 92590
Technical Support: T: 1-800-MILLIPORE (1-800-645-5476) • F: 1-800-437-7502
www.millipore.com

FORMATION OF EMBRYOID BODY:

Mouse ES cells should be grown in proper cell culture conditions for at least 3 passages before attempting to form embryoid bodies. Proper undifferentiated ES cells are characterized by distinct round colonies with a high nucleus to cytoplasm ratio, high alkaline phosphatase levels and distinct phase bright borders.

1. Carefully remove the medium used to culture mouse ES from the 10-cm tissue culture plate and wash the plate twice with 1X PBS.
2. Apply 5 mL Accutase (Catalog No. SCR005) and incubate in a 37°C incubator for 3-5 minutes.
3. Inspect the plate and ensure the complete detachment of cells by gently tapping the side of the plate with the palm of your hand.
4. Apply 5 mL Embryoid Body Formation Medium (pre-warmed to 37°C) to the plate.
5. Centrifuge the tube at 300 xg for 2-3 minutes to pellet the cells.
6. Discard the supernatant.
7. Apply 2 mL Embryoid Body Formation Medium to the conical tube and resuspend the cells thoroughly. **IMPORTANT: Do not vortex.**
8. Count the number of cells using a hemacytometer.
9. Aliquot 2 to 3 x 10^6 cells in 10mL Embryoid Body Formation Medium and place in a sterile 10-cm bacterial Petri dish or ultra low attachment Petri dish. Alternatively, EBs can be formed as a hanging drop and allow to incubate for 2-4 days.
10. Incubate cells in 37°C, 9-10% CO_2 incubator for two days.
11. Using a 10 mL serological pipette, carefully collect the cells (both aggregated EBs and non aggregated cells) into a 15 mL conical tube and let the cells settle by gravity for 15 minutes.
12. Carefully remove supernatant and resuspend the cell pellet in 10 mL fresh Embryoid Body Formation Medium.
13. Transfer the cell suspention to a fresh sterile 10-cm Petri dish or low attachment plate. Incubate in 37°C incubator for 2 days.
14. Further differentiation steps may be performed after the EB's have formed (approximately 4 days).

STORAGE/HANDLING:

The Embryoid Body (EB) Formation Medium should be stored at -20°C until ready to use. Upon thawing, the medium may be stored at 2-8°C for up to one month.

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

28820 Single Oak Drive • Temecula, CA 92590
Technical Support: T: 1-800-MILLIPORE (1-800-645-5476) • F: 1-800-437-7502
www.millipore.com