

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.

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

**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 x g 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 10⁶ 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% CO₂ 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 suspension 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:

SCM018, Embryoid Body (EB) Formation Medium should be stored at -20°C until ready to use. Upon thawing, the basal medium should be stored at 2-8°C and given a 1-month expiration dating.

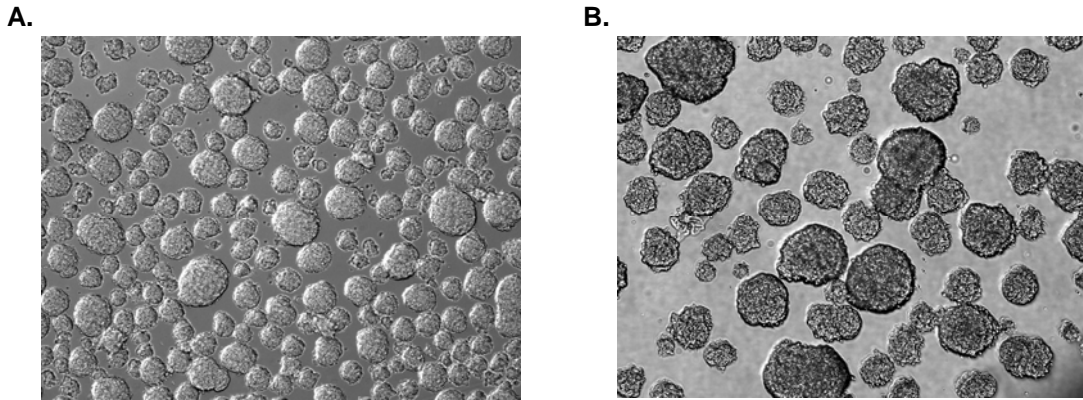
RESULTS:

Figure 1. Representative images of EBs formed by the culture of murine embryonic stem cells (Catalog No. SCR012) in Embryoid Body Formation Medium for **(A)** two days and **(B)** four days.

EBs formed with Embryoid Body Formation Medium can be directed to preferentially differentiate into cardiomyocytes, neurons, and adipocytes.

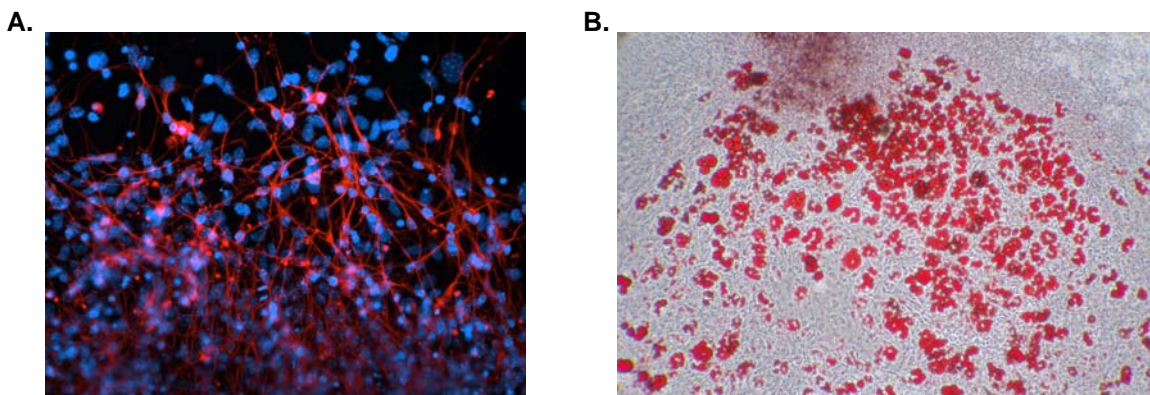


Figure 2. Using the Murine ES Cell Neurogenesis Kit (Catalog. No. SCR101), mouse embryonic stem cells can be differentiated into **(A)** β III-tubulin positive neurons (red). Cell nuclei were counterstained with DAPI (blue) (40X magnification). **(B)** Using the Murine ES Cell Adipogenesis Kit (Catalog. No. SCR100), mouse embryonic stem cells were differentiated into adipocytes as indicated by the accumulation of lipid vacuoles that stain with Oil Red O (10X magnification).

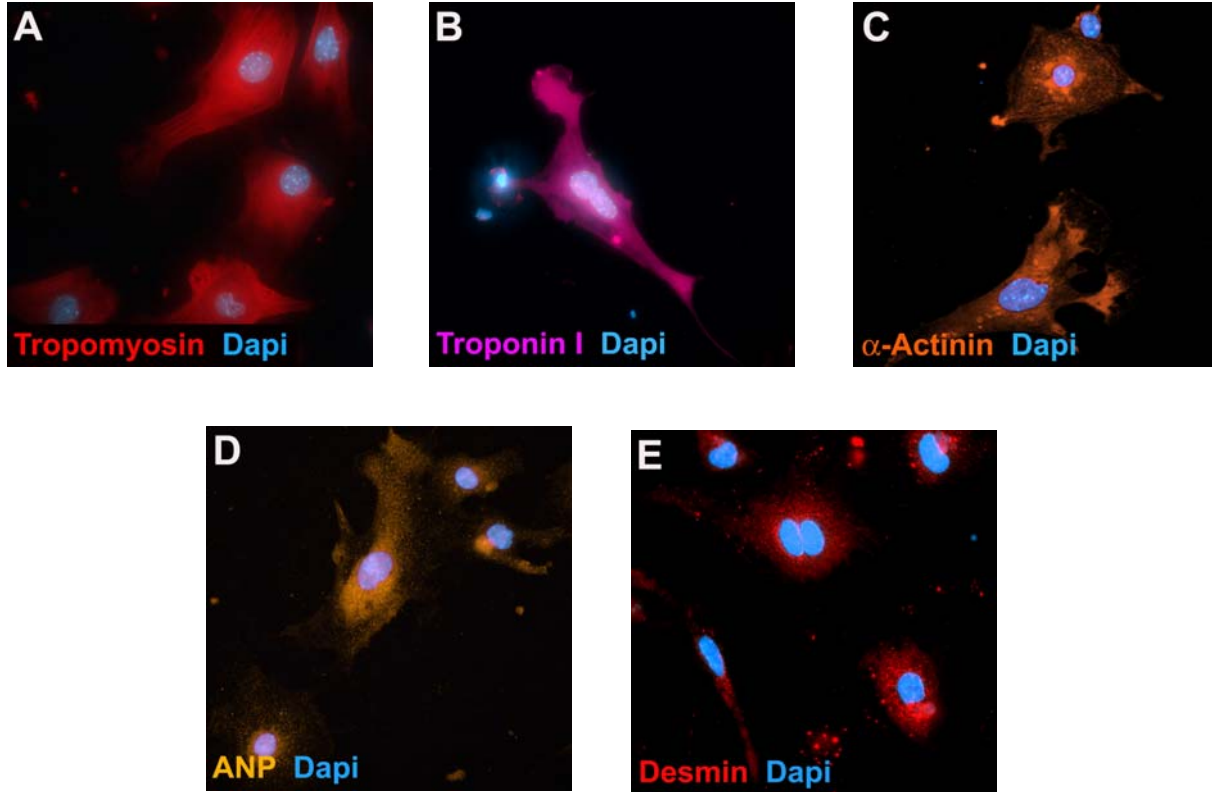


Figure 3: To obtain beating cardiomyocytes, murine ES cells (Catalog No. SCR012) dissociated by Accutase were cultured in hanging drops (1000 cells/drop, 20 μ L/drop) in Embryoid Body Formation Medium (Catalog No. SCM018) to induce embryoid body (EB) formation. After 5 days in a hanging drop culture, the resulting EBs were transferred to a 0.1% gelatin coated 96-well plate at a density of 1 EB/well in fresh Embryoid Body Formation Medium. Individual wells containing beating EBs were identified by eye, trypsinized, pooled and transferred to gelatin coated glass chamber slides in fresh Embryoid Body Formation Medium. After 48 hours of incubation, the slides were fixed with 4% PFA and stained with the indicated cardiomyocyte antibodies (Catalog No. SCR059).

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.*

For research use only; not for use as a diagnostic.

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