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User Manual

OriCell™ Embryoid Body (EB) Formation Medium

Cat. No. MUXES-90051

PRODUCT DESCRIPTION:

Embryonic stem cells (ESCs), derived from the inner cell mass (ICM) of blastocyst-stage embryos, are pluripotent and have a virtually unlimited capacity for self-renewal and differentiation into all cell types comprising all three embryonic germ layers (ectoderm, mesoderm, and endoderm). The formation of embryoid bodies (EBs) is the principal step in the differentiation of ESCs. When maintained in the EB formation medium and in the absence of mouse embryonic fibroblasts (MEF) feeder layers, ESCs differentiate spontaneously and form three-dimensional aggregates. This structure facilitates multicellular interactions in which cell-cell contact exists and gap junctions may be established.

OriCell™ Embryoid Body (EB) Formation Medium has been optimized and qualified to support the formation of EBs. The medium can be used to form EB from hanging drops or suspension culture on non-adhesive Petri dishes.

The product is intended for laboratory research use only. It is not intended for diagnostic, therapeutic, clinical, household, or any other applications.

KIT COMPONENTS:

Embryoid Body (EB) Formation Basal Medium (Cat. No. MUXES-03051)	435 mL
Embryoid Body (EB) Formation-Qualified Fetal Bovine Serum (Cat. No. MUXES-05051)	50 mL
Glutamine	5 mL
Penicillin-Streptomycin	5 mL
Non-essential Amino Acid	5 mL
2-Mercaptoethanol	500 µL

INSTRUCTIONS:

1. Prior to use, thaw the EB Formation-Qualified Fetal Bovine Serum at 2-8°C overnight or until completely thawed. Gently swirl the bottle to ensure homogeneity. The serum has been heat-inactivated and is ready to use after thawing.

***Note*: The thawed serum may contain some flocculent precipitates. The presenceof these substances in serum does not alter the performance characteristics of the product. It is not recommended to filter the serum to remove these precipitates. Doing so may result in the loss of some serum nutrients.**

2. About 30 minutes prior to use, thaw the Non-essential Amino Acid, Penicillin-Streptomycin solution, and Glutamine solution at room temperature. Gently swirl the vials to ensure homogeneity.
3. About 10 minutes prior to use, thaw the 2-Mercaptoethanol at room temperature.

***Note*: Centrifuge the vials briefly at low speed before removing the caps to ensure recovery of the entire content.**

4. Disinfect the external surfaces of the bottles/vials with 70% v/v ethanol for every component in the kit. Allow ethanol to evaporate.
5. Aseptically open the bottles/vials inside a laminar flow hood.
6. Transfer the entire amount of EB Formation-Qualified Fetal Bovine Serum, Non-essential Amino Acid, Penicillin-Streptomycin solution, and Glutamine solution into the EB Formation Basal Medium.
7. Rinse the vials with a small amount of basal medium. Subsequently transfer the rinse medium back into the bottle of basal medium.
8. To transfer the entire amount of 2-Mercaptoethanol, add 0.5 mL of medium to the vial, mix by pipetting, and then transfer the entire mixture back into the bottle of basal medium.
9. Repeat step 8 several times.
10. Gently swirl the fully supplemented (complete) medium to ensure a homogeneous mixture. The complete medium is now ready to use.

***Note*: Although each component in this kit is supplied sterile, it is strongly recommended to filter the fully supplemented (complete) medium.**

FORMATION OF EMBRYOID BODY:

1. Dissociate mouse ESCs by incubating the cells with trypsin solution at 37°C for 1-2 min.
2. Add an appropriate volume of EB Formation Medium (e.g. 3 mL for each well of six-well plate) to stop reaction and gently pipette up and down until the cells in the colonies become single cells.
3. Transfer the cell suspension into a 15 mL conical tube and centrifuge at 250 x g for 5 minutes in order to form a cell pellet.
4. Carefully aspirate as much of the supernatant as possible.
5. Add an appropriate amount of EB Formation Medium to the cell pellet and gently resuspend the cells. Seed the cell suspension in a 100 mm adherent dish.
6. Incubate the adherent dishes in a 37°C incubator for 30-40 minutes to separate MEFs from ESCs.
7. Carefully collect the suspending ESCs. Adjust the cell concentration to 5x105 cells/mL with EB Formation Medium.
8. Seed 10 mL of cell suspension into a 100 mm non-adherent petri dish.
9. Incubate the cells at 37°C inside a 5% CO2 humidified incubator for 5 days to form EBs. Change the medium every other day.
10. Plate EBs into the adherent surface of gelatin-coated tissue culture vessels covered in EB Formation Medium.
11. Incubate the EBs at 37°C inside a 5% CO2 humidified incubator for ~14 days. Change the medium every other day.
12. Stain the differentiated cells with antibodies against endodermal, mesodermal,

and ectodermal markers at day 14 after EB differentiation.

STABILITY AND STORAGE:

All products should be stored in the dark. EB Formation Basal Medium is stable at 2-8°C for up to one year. Other components are stable at -20°C for up to two years.

These products should be discarded beyond the labeled expiration date.

Once prepared, the fully supplemented (complete) medium can be stored for up to one month when stored in the dark at 2-8°C.

For optimal performance, repeated warm-cooling and freeze-thawing should be avoided.

QUALITY CONTROL:

OriCell™ Embryoid Body Formation Medium has been tested for performance on EB formation. The standard evaluation includes:

- Sterility test (bacteria, fungi, and mycoplasma)
- pH test
- Osmolality
- Endotoxin

RELATED PRODUCTS:

Product	Catalog Number
OriCell™ Mouse Embryonic Stem Cell Growth Medium	MUXES-90011

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