Embryoid Body (EB) Formation Medium

Cat No. F- MUXES-9051

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 ES cells. When maintained in the EB formation medium and in the absence of MEF feeder layers, ES cells differentiate spontaneously, and then form threedimensional aggregates. This structure facilitates multicellular interactions, in which cell-cell contact exists and gap juncitons may be established. Embryoid Body (EB) Formation Medium has been optimized and qualified to support the formation of EB. 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, and not for drug, house hold, or other uses.

Kit Components:

Embryoid Body Formation Basal Medium	435mL
Embryoid Body Formation -Qualified Fetal Bovine Serum	50mL
Glutamine	5mL
Penicillin-Streptomycin	5mL
Non-essential Amino Acid	5mL
2-Mercaptoethanol	500μL

Instructions for Use:

① Prior to use, thaw Embryoid Body Formation-Qualified Fetal Bovine Serum under refrigeration (2 to 8°C) over night or until completely thawed. Gently swirl the bottle to ensure homogeneity. Embryoid Body Formation-Qualified Fetal Bovine Serum has been heatinactivated and is ready to use after thawing.

Note: The thawed serum may contain some flocculent precipitates. The presence of 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 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 2-Mercaptoethanol at room temperature.

Note: Centrifuge the vials briefly at low speed (250g) before removing the caps to ensure recovery of entire content.

④ Disinfect with 70% v/v ethanol the external surfaces of the bottles/vials for everycomponent in the kit. Allow ethanol to evaporate away.

⑤ In a laminar flow hood aseptically open the bottles/vials.

⑥ Transfer the entire amount of Embryoid Body Formation -Qualified Fetal Bovine Serum, Non-essential Amino Acid, Penicillin-Streptomycin solution and Glutamine solution into Embryoid Body Formation Basal Medium.

⑦ Rinse the vials with the medium and transfer the rinse medium back to the bottle of basal medium.

⑧ To transfer the entire amount of 2-Mercaptoethanol, add 0.5mL medium to the vials, mix by pipeting and then transfer the

mixture back to the bottle of basal medium as much as Instructions for Use:

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 Embryoid Body (EB) Formation Medium (e.g. 3 ml for each well of six-well plate) to stop reaction and gently pipette up and down until cells in colonies become single cells.

③ Transfer cell suspension into a 15ml conical tube and centrifuge at 250 g for 5 minutes to pellet the cells.

④ Carefully aspirate as much of the supernatant as possible.

⑤ Add appropriate amount of Embryoid Body (EB) Formation Medium to the conical tube and gently resuspend the cells. Plate cell suspension in 100mm adherent dishes.

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 and adjust the cell concentration to 5×105 cells/ml with Embryoid Body (EB) Formation Medium.

⑧ Plate 10ml cell suspension in one 100 mm non-adherent petri dish.

⑨ Incubate the cells at 37°C in a 5% CO2 humidified incubator for 5 days to form EBs, and change the medium every other day.

⑩ Plate EBs into adherent surface of gelatin coated tissue culture vessels in Embryoid Body (EB) Formation Medium.

⑪ Incubate the EBs at 37°C in a 5% CO_2 humidified incubator for about 14 days. Change medium every other day.

⑫ Stain the differentiated cells with antibodies against endodermal, mesodermal and ectodermal markers at day 14 after EB differentiation.

Stability/Storage:

All products should be stored in the dark. Embryoid Body Formation Basal Medium is stable at 2 to 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 to 8°C. For optimal performance, repeated warm-cooling and freeze-thawing should be avoided.

Quality Control:

Embryoid Body (EB) Formation Medium is performance tested on EB formation.

Standard evaluation includes:

1. Sterility test (bacteria, fungi, mold and mycoplasma)
2. pH test
3. Osmolality
4. Endotoxin

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