Embryoid Body Formation Protocol
Adapted from:
Human Embryonic Stem Cells: Laboratory Manual

(Includes Invitrogen product information)

Michal Amit and Joseph Itskovitz-Eldor.

Department of Obstetrics and Gynecology, Rambam Medical Center and The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology

Correspondence to Joseph Itskovitz-Eldor M.D., D.Sc., Department of Obstetrics and Gynecology, Rambam Medical Center, P.O.B. 9602, Haifa 31096, Israel.

Tel.: +972-4-854-2536; Fax: +972-4-854-2503;

E-mail: Itskovitz@rambam.health.gov.il.

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Formation of Embryoid Bodies (EBs):

1. Remove medium from well. Add 0.5 ml splitting medium (see A below), and incubate for at least 30 minutes.

2. Add 1 ml of culture medium (see B below) and gently scrape cells with 5-ml pipette.

3. Collect cell suspension and place into conical tube.

4. Centrifuge 3 minutes at 800 rpm at a recommended temperature of 4 °C.

5. Re-suspend cells in media (see B below) using Gillson 1000 μM tip and plate on 58 mm Petri dish.

6. Add 6 ml of medium.

Note:

If EBs attach to the dish, scrape them off gently .

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A. hES cell splitting medium:

1 mg / ml Collagenase type IV. Invitrogen cat. # 17104

Dulbecco's Modified Eagle's Medium (DMEM). Invitrogen cat #11960.

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B. hES cell media:

B.1 Normal medium:

Final concentrations:

80% Dulbecco's Modified Eagle's Medium (DMEM). Invitrogen cat #11960 or Knockout DMEM KO-DMEM. Invitrogen cat. # 10829.

20%Fetal Bovine Serum defined (FBSd). Invitrogen cat. # 16141

1% Non essential amino acids. Invitrogen cat. # 11140

mM L-glutamine Invitrogen cat. # 21051

0.1 mM β-Mercaptoethanol Invitrogen, cat. # 21985

Preparation:

1. Pour all materials into 22 μM filter unit, and filter.

2. Store at 4^{o}C.

B.2 Serum free medium:

Final concentrations:

80% KO-DMEM Invitrogen cat. # 10829.

20% Serum replacement (SR) Invitrogen cat. # 10828.

1% Non essential amino acids. Invitrogen cat. # 11140

0 mM L-glutamine. Invitrogen cat. # 21051

0.1 mM β-Mercaptoethanol. Invitrogen, cat. # 21985

4 ng/ml basic Fibroblasts Growth Factor (bFGF). Invitrogen cat. # 13256

Preparation:

1. Pour all materials into 22 μM filter unit, and filter.

2. Store at 4^{o}C .

May be used within two weeks of preparation.

