

**Title: Transfer of Mouse Embryonic Stem Cell Line E14 (ES-E14TG2a)(ATCC CRL-1821)  
to Non-binding Petri Plates to Form Embryoid Bodies**

**Approvals:**

Preparer: W. H. Woodruff Date 6 February 2015  
Reviewer: Maggie Bryans Date 12 July 2016

1. Purpose: This SOP describes the techniques and materials needed to initiate the formation of embryoid bodies from E14 mESC monolayer.
2. Scope: This SOP will enable any qualified technician to successfully produce embryoid bodies from any appropriate mESC line.
3. Responsibilities:
  - 3.1. It is the responsibility of the course instructor /lab assistant to ensure that this SOP is performed as directed and to update the procedure when necessary.
  - 3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. References:
  - 4.1. SOP: ID# REA-006 Preparation of DMEM-HG Stock Reagent
  - 4.2. SOP: ID# REA-008 Preparation of mESC Differentiation Media
  - 4.3. SOP: ID# REA-002 Preparation of 1% and 0.25% Trypsin Stock Reagents
  - 4.4. SOP ID# REA-004 Preparation of Dulbecco's Phosphate Buffered Saline
  - 4.5. SOP ID# BTC-010 Preparation of the Laminar Flow Safety Cabinet, Class II, for Aseptic Cell Culture Protocols
  - 4.6. SOP ID# BTC-011 Set Up and Operation of the Olympus Inverted Microscope for Cell Viewing
  - 4.7. SOP ID# BTC-012 Set Up and Operation of the EVOS Inverted Microscope for Cell Viewing
5. Definitions:
  - 5.1. mESC Embryoid Bodies (EBs): spherical structures formed by mESCs grown in suspension. The EBs are the pre-stage to differentiation into a variety of cell types.
6. Precautions:
  - 6.1. All activities must be carried out using aseptic techniques and wearing PPE.
  - 6.2. No other precautions are noted for this protocol.
7. Materials:
  - 7.1. Sterile 2 ml, 5 ml and 10 ml pipettes

- 7.2. Auto pipette aid
- 7.3. Sterile, non-tissue culture petri plate
- 7.4. 400 ml beaker for waste and discard liquids
- 7.5. 100 ml mESC Differentiation Media (see SOP)
- 7.6. 100 ml Dulbecco's Phosphate Buffered Saline (D-PBS) (see SOP)
- 7.7. 1 ml 0.25% trypsin (see SOP)
- 7.8. 70 -80% confluent healthy E14 culture

## 8. Procedure:

### 8.1 General

- 8.1.1. Gather and inventory all the required materials.
- 8.1.2. Prepare the Laminar Flow Safety Cabinet for aseptic culture work (see SOP)
- 8.1.3. Disinfect with 75% ethanol (spray and wipe) and place all required materials into the Laminar Flow Safety Cabinet

### 8.2. Dissociating the cells from the flask surface

- 8.2.1. Obtain an E14 culture from the incubator and examine using the inverted microscope. Check for good morphology, no excess turbidity (sign of contamination), ~70 - 80% confluency and degree of floating, rounded cells. A small amount is an indication of cells dividing; large numbers are often an indicator of excessive cell death.

- 8.2.2. Aseptically remove the culture media in the flask and discard in the waste beaker.

NOTE: gently "sloshing" the flask before removing the spent culture media will help increase the viability of your culturing by ensuring the dead cells are loose and floating for easier removal.

- 8.2.3. Rinse the cell monolayer with 5 ml of D-PBS.

- 8.2.3.1. Add 5 ml of D-PBS to the flask
- 8.2.3.2. "Swirl" the flask to ensure rinsing in all corners and edges
- 8.2.3.3. Remove the D-PBS to the waste beaker

- 8.2.4. Repeat the rinse step (8.2.3.) once

- 8.2.5. Add 1 ml of 0.25% trypsin to the now empty T-25 flask with the C2C12 cells

- 8.2.5.1. Carefully swirl to ensure the entire cell layer is covered with trypsin

- 8.2.6. Place the flask into the 37°C incubator

8.2.6.1. Check the cells for dissociation after 4 – 5 minutes by looking at them under the inverted microscope. Dissociated cells will be rounded and many will be floating. If the majority of the cells are not yet rounded return the flask to the incubator and check again in 3 minutes. Continue this process until at least 80% of the cells are rounded and/or floating.

8.2.7. When ready, gently rap the flask against the palm of your hand to dislodge the rounded cells from the surface of the flask into a single cell suspension.

8.2.8. Quickly add 4 ml of E14 Differentiation Media with 10% FBS to the 1 ml of trypsin / Cell suspension.

NOTE: the FBS in the media will inactivate the protease activity of the trypsin

8.2.9. Using a 5 ml pipette, triturate the cells by gently drawing up and expelling the cells while loosely holding the pipette in a corner of the flask. The goal is to achieve a single cell suspension without excess damage to the cells.

TIP: Do not draw up nor expel all of the liquid. Leaving ~ 0.5 ml in the flask or in the pipette will greatly reduce the amount of foam generated.

TIP: At this point, a check under the inverted microscope should reveal an ~95% single cell suspension.

8.3. Distribution of suspended E14 cells to a petri plate

8.3.1. Prepare culture vessels

8.3.1.1. Using a Sharpie, label petri plate with the cell line, the growth media used, the date and your initials

8.3.1.2. Add 5 ml of E14 differentiation media to the labeled petri plate

8.3.2. Make sure the cells in the flask are well suspended by gently shaking the flask. Transfer the entire 5 ml of the E14 cell suspension to the prepared petri plate.

8.4. You may optionally continue growing pluripotent (i.e., undifferentiated) E14 cells in the original (mother) vessel by adding 6 ml of E14 Pluripotent media back into the vessel.

NOTE: Pluripotent media differs from differentiation media by the addition of 1000 U/ml leukemia inhibitory factor (LIF).

8.3.4. Return all vessels to the incubator.

8.3.5. Check all cultures for contamination after 24 hours.

10. History:

Name	Date	Amendment
W.H. Woodruff	20 June, 2015	Initial release
W.H. Woodruff	15 July, 2016	Minor changes to the wording for clarity

NOTE: NOTE: The following information is not usually found on an SOP but because this is a teaching instrument, I have included it here for completeness.

#### Formulation of mESC (E14) Differentiation Media

Dulbecco's Modified Essential Media, High Glucose	90 ml
100X stock Sodium Pyruvate (1 uM final)	1 ml
100X stock non-essential amino acids 0.1 uM final)	1 ml
100X stock L-Glutamine (2 mM final)	1 ml
beta-2-mercaptoethanol (0.1 uM final)	0.7 ul
Fetal Bovine Serum (10% final)	10 ml

To prepare mESC (E14) Pluripotent media add Leukemia Inhibitory Factor (LIF) to a final concentration of 1000 U/ml