Alamance Community College
1247 Jimmie Kerr Rd
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Title: Preparation of ES-E14TG2a (E14) Stem Cell Pluripotent Media (PPM) (with a note on the preparation of Differentiation Media)

Approvals:

Preparer: W. H. Woodruff Date 12 June 2016
Reviewer: Maggie Bryans Date 12 July 2016

1. Purpose: To provide instruction for the preparation of 100 ml of PPM growth media and/or differentiation media.

2. Scope: This SOP applies to qualified technicians preparing media for the growth, maintenance and differentiation of E14 stem cell culture. It is recommended that this SOP be executed at least one day prior to the media being used.

3. Responsibilities:
   - 3.1. It is the responsibility of the lab supervisor to ensure that this SOP is performed as directed and to update the procedure when necessary.
   - 3.2. It is the responsibility of the technician to follow the SOP as described and to inform the supervisor about any deviations or problems that may occur while performing the procedure.

4. References:
   - 4.1. SOP ID#: Preparation of Dulbecco's Modified Eagles Media – High Glucose
   - 4.2. SOP ID#: Preparation of Penicillin / Streptomycin Stock Solution
   - 4.3. SOP ID#: Disinfecting Laminar Flow Safety Cabinet for Aseptic Cell Culture Use

5. Definitions:
   - 5.1. None needed

6. Precautions:
   - 6.1. Always wear the appropriate Personal Protective Equipment (PPE)

7. Materials:
   - 7.1. Stock Dulbecco's Modified Eagles Media – High Glucose (DMEM-HG) (Sigma catalog # D1152)
   - 7.2. Sterile Fetal Bovine Serum (FBS)
   - 7.3. Stock Penicillin / Streptomycin (pen/strep) vial from -20^{o}C freezer, thawed
   - 7.4. Sodium Pyruvate (Sigma catalog #)
   - 7.5. Non-Essential Amino Acids (Sigma catalog #)
   - 7.6. Beta-2-mercaptoethanol (Sigma catalog #)
   - 7.7. Leukemia Inhibitory Factor (LIF) (Thermo-Fisher catalog #)
   - 7.5. Sterile media storage bottle, 100 ml – 250 ml capacity
   - 7.6. Sterile 2 ml, 5 ml, 10 ml and 25 ml serological pipettes

7.7. Drummond pipette aid
7.8. 75% ethanol in spray bottle plus wipe

8. Procedure:

8.1 General

8.1.1. Prepare a laminar flow safety cabinet for aseptic use following the SOP
8.1.2. Gather and inventory all of the materials listed
8.1.3. Spray all materials with 75% ethanol and place in laminar flow safety cabinet

8.2. Preparing 100 ml of E14 PPM growth media

8.1.1. Use aseptic techniques for all of the following steps in the laminar flow safety cabinet
8.2.2. Transfer 88 ml of the stock DMEM-HG media to the sterile media storage bottle
8.2.3. Add 1 ml of the sodium pyruvate to the stock DMEM-HG
8.2.4. Add 1 ml of non-essential amino acids to the stock DMEM-HG
8.2.5. Add 0.7 ul (microliters) of beta-2-mercaptoetahnol to the stock DMEM-HG
8.2.6. Add LIF to a final concentration of 10 ng / ml of PPM media
NOTE: Omit the LIF to prepare E14 Differentiation media
8.2.6. Transfer 10 ml of the FBS into the sterile media storage bottle
8.2.7. transfer the 1 ml stock pen/strep solution to sterile media storage bottle
NOTE: final concentrations: Pen = 100 IU/ml & Strept = 100 mg/ml
8.2.8. Mix the contents by swirling gently; avoid excess foam

8.3. Test for sterility before using

8.3.1. Place the tightly capped media in a 37^{o}C incubator
8.3.2. Check for contamination at 24 to 48 hours
8.3.3. If contaminated, discarded and repeat procedure; if not contaminated, use for culture maintenance

8.4. Storage

8.4.1. Store unused portions in a 4^{o}C cabinet

9. Document History:

Name	Date	Amendment
William H Woodruff	12/6/2015	Initial release