PREPARATION OF MOUSE EMBRYONIC FIBROBLAST (MEF) CULTURE MEDIUM

OBJECTIVE: Mouse Embryonic Fibroblasts (MEFs) are used to support the growth of human Embryonic Stem Cells (hESCs). Defined complete medium is needed for successful growth of derived MEFs. This Standard Operating Procedure (SOP) details how to prepare complete medium for the culture of MEFs.

SCOPE: This procedure applies to all Massachusetts Human Stem Cell Bank laboratory personnel responsible for culture of MEFs.

RESPONSIBILITY: It is the responsibility of the Laboratory Operations Manager and Quality Assurance Officer to ensure all laboratory personnel are properly trained in and follow this SOP.

SAFETY: All laboratory personnel should be in compliance with UMASS Employee Health and Safety regulations when working in the laboratory. Protective equipment, such as a lab coat and disposable gloves must be worn when working in the lab.

ABBREVIATIONS AND DEFINITIONS
MEF: Mouse Embryonic Fibroblast
hESCs: Human Embryonic Stem Cells
HI-FBS: Heat-Inactivated Fetal Bovine Serum
NEAA: Non-Essential Amino acid
DMEM: Dulbecco's Modified Eagle Medium
SOP: Standard Operating Procedures
UMASS: University of Massachusetts Medical School

REFERENCES
Not applicable

1. MATERIALS REQUIRED

1.1. EQUIPMENT

- Pipette-aid
- Sterile biosafety cabinet (tissue culture hood)

1.2. SUPPLIES

- 5 ml sterile serological pipettes (Costar 4487)
- 10 ml sterile serological pipettes (Costar 4488)
- 25 ml sterile serological pipettes (Costar 4489)
- 50 ml centrifuge tubes (BD Falcon 352098)
- 500 ml bottle connected with a 0.22 μM Stericup (Millipore SCGPU05RE)
- Black extra-fine alcohol proof pen (VWR 52877-150)

- Disposable nitrile gloves (World Wide Medical Supplies 71011000-3)

1.3. REAGENTS

- DMEM-liquid (Invitrogen 11965-118)
- MEM Non-Essential Amino Acid Solution (Invitrogen 11140-050)
  Heat Inactivated Fetal Bovine Serum -HI-FBS (Invitrogen 16000-069)
- 70% ethanol (Diluted from 95% ethanol, Fisher NC9608803)

2. PREPARATION

2.1 THAW HI-FBS IF NEEDED

1. Check refrigerator for HI-FBS aliquots. If there are no HI-FBS aliquots or a bottle of HI-FBS available in the refrigerator, go to freezer for an aliquot and thaw using either method below. If the frozen aliquots in the freezer are low, notify appropriate personnel.
   - **Option one:** Thaw HI-FBS overnight in a 4°C refrigerator. If HI-FBS is not completely thawed the next day, put it in a 37°C water bath.
   - **Option two:** Thaw HI-FBS directly in a 37°C water bath. It will take 2-3 hours to thaw a 500 ml bottle. Gently swirl the bottle frequently to accelerate the process.

2.2. DOCUMENTATION

1. From the binder containing the current Reagents/Media Preparation Log Sheets, take out the **MEF Culture Media Preparation Log Sheet**.
2. Assign a batch number to the medium to be prepared as: MEF.M-yy-mm-dd. For example, for Culture Media prepared on 10/01/2008, the batch# will be: MEF.M-08-10-01.
3. Record the batch# number, date, initials, and the volume to be prepared on the Log Sheet.
4. Take the Log Sheet to the lab for recording of other information.

2.3. PREPARE THE BIOSAFETY CABINET (TISSUE CULTURE HOOD)

1. Make sure there are sufficient amounts of materials listed below near the hood:
   - 5 ml, 10 ml, 25 ml sterile serological pipettes
   - Absorbent paper towels (or Kimwipes)
   - 70% ethanol spray
   - Appropriate-size disposable gloves
   - Appropriate-size filter unit

3. PROCEDURE

3.1. STERILIZATION PREPARATION BEFORE STARTING WORK IN THE HOOD

1. Wash hands and arms thoroughly (about one minute) with soap.
2. Rinse completely with warm tap water.
3. Dry hands and arms with paper towel.

4. Put on appropriate-size gloves.
5. Spray 70% ethanol on the gloves and a paper towel until fully saturated.
6. Thoroughly clean the working surface in the hood with the ethanol-sprayed paper towel. Make sure the following items are in the hood:
   - 50 ml sterile centrifuge tubes
   - Black extra-fine alcohol proof pen

3.2. ALIQUOT THE HI-FBS IF A WHOLE BOTTLE WAS THAWED

1. Label sterile 50 ml tubes in the hood as:
   - HI-FBS
   - Vendor
   - Vendor's cat# and lot #
   - Expiration date
   - Initials
2. Transfer the thawed HI-FBS bottle to hood after thoroughly cleaning the outside with 70% ethanol.
3. Leave enough HI-FBS in the bottle to be used in **Section 3.3** (Leave the bottle in the hood if it is to be used shortly, or store the bottle in a 4°C refrigerator). Pour the remainder into the labeled 50 ml tubes in 40 ml aliquots.
4. Store the aliquots in a -20°C freezer.

3.3. PREPARATION OF MEF CULTURE MEDIA

1. In the hood, open a 500 ml filter and bottle unit. Label the bottle as:
   - MEF medium batch number as MEF.M-yy-mm-dd
   - Expiration date (mm-dd-yyyy, 14 days after media preparation). For example: Exp: 07-10-2008
   - Initials
2. From the refrigerator, take DMEM, HI-FBS, NEAA (if not in the hood) and place them in the hood after thoroughly cleaning with 70% ethanol.
3. According to the final total volume, add the appropriate amount of the following ingredients to the 500 ml filter cup as shown in the table below:

Ingredient	100 ml total	250 ml total	500 ml total
DMEM	70 ml	225 ml	450 ml
HI- FBS	90 ml	25 ml	50 ml
NEAA	1 ml	2.5 ml	5 ml

Note 1: The DMEM may be measured by pouring directly into the graduated filter cup of the filter/bottle unit. The other solutions should be added using serological pipettes.

Note 2: Scale up or down proportionately if another quantity of medium is needed.

4. Filter the media through the Stericup filter into the attached bottle.
5. Discard the filter unit. Tightly cap the bottle containing MEF medium.
6. Store the medium bottle at 4°C, and use the medium within 14 days.

7. Record all necessary information (e.g., lot numbers, expiration dates) in the **MEF Medium Preparation Log Sheet**. Sign and store it in the appropriate binder.

MEF CULTURE MEDIUM PREPARATION LOG SHEET

Note: Medium batch# is assigned as: MEF.M-yy-mm-date.

			Culture media			Manufacturer's information		
Date:	Prep by:	Reviewed by:	Batch#	Volume		DMEM	HI FBS	NEAA
					Lot#			
					Expiration Date			
					Lot#			
					Expiration Date			
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