SOP: **Propagation of H7 hESC**
Date modified: **12/17/09**
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Ordering Information

H7 undifferentiated human embryonic stem cells (H7-hESC) can be ordered as frozen ampoules from the WiCell Research Institute through the National Stem Cell Bank:

Name: WA07 (H7) – human embryonic stem cells

Materials List

Reagent	Vendor	Catalog Number
DMEM/F12 + glutamine	Gibco/Invitrogen	11320
DMEM + glutamine	Gibco/Invitrogen	11995
Knockout Serum Replacement	Gibco/Invitrogen	10828
Fetal Bovine Serum	Hyclone	SV30014
Sodium Pyruvate	Gibco/Invitrogen	11360
Non-essential amino acids	Gibco/Invitrogen	11140
Beta-Mercaptoethanol	Gibco/Invitrogen	21985-023
Penicillin/Streptomycin	Gibco/Invitrogen	15070
Matrigel (GFR)	BD	356230
DNase	Calbiochem	260913
Collagenase IV	Gibco/Invitrogen	17104-019
Trypsin (0.05%)-EDTA	Gibco/Invitrogen	25300
Basic fibroblast growth factor	Peprotech	AF-100-18B

Procedure

A. Subculturing and maintenance of undifferentiated H7-hESC cells on Matrigel (10cm plate format)

1) Aspirate media and add 5mL of Collagenase IV (200 U/mL)
2) Incubate for 5-7 min in 37°C incubator (watch for cells to start lifting off plate)
3) Aspirate collagenase
4) Add 5 mL of 0.05% Trypsin-EDTA
5) Incubate for 10 seconds at RT
   *Monitor cells on scope: want cells to round up but remain adherent
6) Aspirate Trypsin
7) Add 1mL hES Stop Solution (1:1 of FBS:DMEM/F12) plus 1:800 Matrigel and 200 U/mL DNase
8) Scrape cells gently and evenly with rubber scraper
9) Resuspend with 5 mL pipette
   **Hold pipette tip on bottom of dish to disperse clumps
10) Transfer cells to 14 mL snap cap tube(s)
11) Rinse with 5 mL hES Wash Medium (DMEM/F12 + P/S)
12) Pellet cells at 800 rpm x 5 min
13) Carefully aspirate supernatant down to pellet
14) Resuspend cells in MEF-CM supplemented with 8 ng/mL bFGF
15) Split cells 1:6 on Matrigel-coated plates
16) Cells are grown in 37°C/5% CO2 incubator with daily media changes. Cells should be passaged when ~70% confluent (4-6 days, depending on cell batch).

B. Production of mouse embryonic fibroblast conditioned medium (MEF-CM)

1) Plate 13 x 106 irradiated P4 MEFs on a T225 flask in MEF medium (DMEM with 10% FBS, 1% penicillin/streptomycin)
2) After cells have plated (~4 hours), add 60 mL pre-conditioned medium*
3) After 24 hours, collect conditioned medium and replace with fresh pre-conditioned medium
4) Collect conditioned medium for 7 consecutive days
5) Pool all collected conditioned medium and sterile-filter

*Pre-conditioned medium consists of DMEM/F12 + glutamine, 20% Knock-out serum replacement, 1% sodium pyruvate, 1% non-essential amino acids, 1% penicillin/streptomycin and 0.1mM beta-mercapthoethanol. Just prior to conditioning, bFGF is added at a concentration of 4 ng/mL.

C. Harvest

1) Passage cells until the desired cell number is achieved
2) Remove cells from flasks according to protocol described above under 'subculturing'
3) Examine viability using Trypan blue staining (SOP TP-7)