

SOP: Propagation of H7 hESC
Date modified: 12/17/09
Modified by: S. Paige/S. Hansen (UW)

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	Peptotech	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 13×10^6 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 0.1 mM 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)