

Materials List for:

Robust Generation of Hepatocyte-like Cells from Human Embryonic Stem Cell Populations

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Materials

Matrigel coating plates and flasks

1. Matrigel (10 mL, BD Biosciences, UK); store at -20°C.
2. KO-DMEM (500 mL, Gibco, Invitrogen, UK); store at 4°C.
3. Tissue culture plates (6 well, 12 well, Corning, UK)
4. Tissue culture flask (25 cm² vented, Corning, UK)

hESC Maintenance

1. Mouse embryonic fibroblast conditioned medium (MEF-CM) (100 mL, R & D Systems, USA); store at -20°C.
2. BSA solution (50 mL, Sigma Aldrich, UK); store at 4°C.
3. Human basic fibroblast growth factor (100 µg, Peptide, USA); store at -20°C.

Passaging hESCs with collagenase

1. Confluent well or flask of hESCs.
2. Matrigel coated wells or flasks as appropriate.
3. Phosphate buffered saline (-MgCl₂, -CaCl₂) (500 mL, Gibco, Invitrogen, UK); store at room temperature.
4. Collagenase IV (1 g, Gibco, Invitrogen, UK); store at 4°C.
5. Mouse embryonic fibroblast conditioned medium (MEF-CM) (100 mL, R & D Systems, USA).
6. Human basic fibroblast growth factor (100 µg, Peptide, USA).

Differentiation of hESCs to hepatic endoderm

1. RPMI 1640 (500 mL, Gibco, Invitrogen, UK); store at 4°C.
2. B27 Supplement (10 mL, Gibco, Invitrogen, UK); store at -20°C.
3. Activin A (2 µg, Peptide, USA); store at -20°C.
4. Recombinant mouse Wnt3a (2 µg, R & D Systems, USA); store at -20°C.
5. KO-DMEM (500 mL, Gibco, Invitrogen, UK); store at 4°C.
6. KO-SR (500 mL, Gibco, Invitrogen, UK); store at -20°C.
7. Non-essential amino acids (100 mL, Gibco, Invitrogen, UK); store at 4°C.
8. β-Mercapto-ethanol (10 mL, Gibco, Invitrogen, UK); store at 4°C.
9. DMSO (Sigma Aldrich, UK); store at room temperature
10. Leibovitz L-15 culture medium (500 mL, Sigma Aldrich, UK); store at 4°C.
11. Tryptose phosphate broth (100 mL, Sigma Aldrich, UK); store at 4°C.
12. F-tal bovine serum, heat inactivated (500 mL, Gibco, Invitrogen, UK); store at -20°C.
13. Hydrocortisone 21-hemisuccinate (100 mg, Sigma Aldrich, UK); store at -20°C.
14. Insulin (bovine pancreas) (100 mg, Sigma Aldrich, UK); store at -20°C.
15. L-Glutamine (100 mL, Gibco, Invitrogen, UK); store at -20°C.
16. Ascorbic acid (25 g, Sigma Aldrich, UK); store at -20°C.
17. Human HGF (10 µg, Peptide, USA); store at -20°C.
18. Recombinant Human Oncostatin M (OSM) (50 µg, R & D Systems, USA); store at -20°C.
19. Syringe driven filter unit 0.22 µm (Millipore, UK)

Characterisation of hESC derived Hepatic Endoderm

Immunostaining

1. Phosphate buffer saline (-MgCl₂, -CaCl₂) (500 mL, Gibco, Invitrogen, UK); store at room temperature.
2. PBST, PBS made up with 0.1% TWEEN 20 (Sigma-Aldrich, UK).
3. Paraformaldehyde (PFA) (Sigma-Aldrich, UK) is made up in PBS, store -20°C.
4. Glycerol (Sigma-Aldrich, UK), store at room temperature.
5. Tris Base (Sigma-Aldrich, UK), store at room temperature.
6. Ethanol
7. Serum (AbD Serotech, UK), store at -20°C.
8. Secondary Antibody, Alexa Fluorophores (Molecular Probes, Invitrogen, UK).

9. MOWIOL 4-88 (Polysciences Inc, USA) is made up in Tris HCL and glycerol as per manufacturers instructions. DAPI (Pierce, Thermo Fisher Scientific, UK) is added to the MOWIOL solution at a 1:1000 dilution.

Primary antibodies			
Antigen*	Type	Supplier	Dilution
ALB	Mouse Monoclonal	Sigma Aldrich	1/500
E-Cadherin	Mouse Monoclonal	Millipore	1/100
α-fetoprotein	Mouse Monoclonal	Sigma	1/500
SSEA-4 FITC	Mouse Monoclonal	Biologend	1/100
IgG	Mouse Monoclonal	DAKO	1/500
Secondary antibodies			
Anti-mouse FITC conjugate	Goat Monoclonal	Invitrogen	1/400

Table 2. The antibodies used for hESC derived hepatic endoderm immunostaining, the concentrations used, the species developed in and the companies they are purchased from.

Functional Analysis of Hepatic Endoderm and Normalisation (per mg protein)

Cytochrome P450 Assays

1. p4-GLO CYP3A4, CYP1A2, Kits and luminometer (Promega, USA).
2. White flat bottom 96 well assay plate (BD Biosciences, UK).
3. BCA Assay Kit (Pierce, Thermo Fisher Scientific, UK).
4. Transparent 96 well assay plate (IWAKI, UK)