

Materials List for:

Induced Differentiation of M cells in Human Stem Cell-Derived Ileal Enteroid Monolayers

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Materials

Name	Company	Catalog Number	Comments
[Leu15]-Gastrin I	Sigma-Aldrich	G9145	MCMGF+ and DM ingredient Solvent: PBS Stock Concentration: 10 µM Final Concentration: 10 nM
0.5M EDTA	Invitrogen	15575020	For breaking up ECM Solvent: PBS Stock Concentration: 0.5 M Final Concentration: 0.5 mM
40 µm cell strainer	Corning	352340	For excluding clumps from single cells Solvent: Stock Concentration: Final Concentration:
A-8301	Sigma-Aldrich	SML0788-5MG	MCMGF+ and DM ingredient Solvent: DMSO Stock Concentration: 500 µM Final Concentration: 500 nM
Advanced DMEM/F12	Invitrogen	12634-028	MCMGF+ and DM Basal medium Solvent: Stock Concentration: Final Concentration:
Alexa Fluor 594 goat anti-mouse IgG	Thermo Fisher	A-11005	For secondary stain Solvent: Stock Concentration: Final Concentration: 1:200
Alexa Fluor 647 Phalloidin	Invitrogen	A22287	Optional secondary stain for F-actin Solvent: Stock Concentration: Final Concentration: 1:100
B27 Supplement	Invitrogen	17504-044	MCMGF+ and DM ingredient Solvent: Stock Concentration: 50x Final Concentration: 1x
Bovine Serum Albumin	Chem-Impex	00535	5% for blocking solution Solvent: PBS Stock Concentration: Final Concentration: 0.01
Chloroform	Fisher Scientific	C298-500	For RNA isolation Solvent: Stock Concentration: Final Concentration:

Circle coverslips	Thomas Scientific	1157B50	For mounting membrane on glass slide Solvent: Stock Concentration: Final Concentration:
DAPI (4',6-diamidino-2-phenylindole)	Thermo Fisher	62247	For secondary stain Solvent: PBS Stock Concentration: 100x Final Concentration: 1x
DEPC Treated RNase free H2O	Fisher Scientific	BP561-1	For RNA isolation Solvent: Stock Concentration: Final Concentration:
DNA Removal Kit	Invitrogen	AM1906	For RNA isolation Solvent: Stock Concentration: Final Concentration:
Ethyl Alcohol, 200 proof	Sigma Aldrich	EX0276-4	For RNA isolation Solvent: Stock Concentration: Final Concentration:
Feather Scalpels	VWR	100499-580	For cutting membrane from transwells Solvent: Stock Concentration: Final Concentration:
Fetal Bovine Serum (FBS)	Gibco	26140079	For inactivating trypsin Solvent: Advanced DMEM/F12 Stock Concentration: 1 Final Concentration: 0.1
Glass slides	Mercedes Scientific	MER 7200/90/WH	For mounting membrane on glass slide Solvent: Stock Concentration: Final Concentration:
GlutaMAX	Invitrogen	35050-061	MCMGF+ and DM ingredient Solvent: Stock Concentration: 200 mM Final Concentration: 2 mM
GP2 Antibody	MBL International	D277-3	Surface stain for M cells Solvent: Stock Concentration: Final Concentration: 1:100
HEPES	Invitrogen	15630-080	MCMGF+ and DM ingredient Solvent: Stock Concentration: 1 M Final Concentration: 10 mM
Human Serum IgA	Lee BioSolutions	340-12-1	For functional analysis of M cells Solvent: PBS Stock Concentration: 1 mg/mL Final Concentration: 10 µg
L-Wnt3a conditioned media	Cell line from ATCC	CRL-2647	Refer to ATCC Product Sheet for L Wnt3A (ATCC CRL2647) for conditioned media protocol; MCMGF+ ingredient Solvent: Stock Concentration: Final Concentration: 75% in MCMGF+ 0% in DM
Matrigel, GFR, phenol free	Corning	356231	Extracellular Matrix (ECM) Solvent:

			Stock Concentration: Final Concentration:
Mouse recombinant EGF	Invitrogen	PMG8043	MCMGF+ and DM ingredient Solvent: PBS Stock Concentration: 50 µg/mL Final Concentration: 50 ng/mL
N2 Supplement	Invitrogen	17502-048	MCMGF+ and DM ingredient Solvent: Stock Concentration: 100x Final Concentration: 1x
N-acetylcysteine	Sigma-Aldrich	A9165-5G	MCMGF+ and DM ingredient Solvent: H2O Stock Concentration: 500 mM Final Concentration: 1 mM
Noggin conditioned media	Cell line gift from Dr. Gijs van den Brink (University of Amsterdam)		Ref 30 for conditioned media protocol; MCMGF+ and DM Ingredient Solvent: Stock Concentration: Final Concentration: 5% in MCMGF+ 5% in DM
Paraformaldehyde (PFA)	MP Biomedicals	2199983	For fixing monolayers Solvent: PBS Stock Concentration: 0.16 Final Concentration: 0.04
PBS, -Mg, -Ca	Corning	MT21040CV	Solvent for 0.5 mM EDTA Solvent: Stock Concentration: Final Concentration:
Penicillin/Streptomycin	Invitrogen	15140-122	Optional ingredient of MCMGF+ and DM Solvent: Stock Concentration: 100x Final Concentration: 1x
Prolong Gold	Invitrogen	P36930	Antifade mounting solution Solvent: Stock Concentration: Final Concentration:
Qiagen RNeasy Kit	Qiagen	74106	For RNA isolation Solvent: Stock Concentration: Final Concentration:
Recombinant human RANKL	Peprtech	310-01	Used to induce M cells Solvent: H2O Stock Concentration: 0.1 mg/mL Final Concentration: 200 ng/mL
Recombinant murine TNFa	Peprtech	315-01A	Used to induce M cells Solvent: H2O Stock Concentration: 5 mg/mL Final Concentration: 50 ng/mL
R-spondin conditioned media	Cell line from Trevigen	3710-001-01	Refer to Trevigen Cultrex Rspo1 Cells product manual (HA-R-Spondin1 293T cell line) for conditioned media protocol; MCMGF+ Ingredient Solvent: Stock Concentration: Final Concentration: 10% in MCMGF+ 0% in DM
Secondary anti-human IgA antibody	Jackson Immuno Research	109-545-011	For secondary stain Solvent:

			Stock Concentration: Final Concentration: 1:200
Super Script IV Reverse Transcriptase	Thermo Fisher	18091200	For conversion of RNA to DNA Solvent: Stock Concentration: Final Concentration:
Transwell inserts, 24 well-sized	Greiner Bio-One	662641	0.4 µm pore size Solvent: Stock Concentration: Final Concentration:
TritonX-100	Sigma-Aldrich	T8787	Not required during GP2 primary stain Solvent: 1% BSA Stock Concentration: Final Concentration: 0.001
TRIzol	Invitrogen	15596018	For RNA isolation Solvent: Stock Concentration: Final Concentration:
TrypLE Express	Invitrogen	12605010	Trypsin for breaking up enteroids into single cells Solvent: Stock Concentration: Final Concentration:
Y-27632	Sigma-Aldrich	Y-0503	MCMGF+ ingredient on day 0 Solvent: H2O Stock Concentration: 5 mM Final Concentration: 10 µM
GAPDH forward primer			CATGAGAAGTATGACAACAGCCT
GAPDH reverse primer			CGTTTCCCGCAAGACGTAAC
GP2 forward primer			CAATGTGCCTACCCACTGGA
GP2 reverse primer			ATGGCACCCACATACAGCAC
LYZ forward primer			CGCTACTGGTGTAAATGATGG
LYZ reverse primer			TTTGACAAGCTACAGCATC
MUC2 forward primer			ATGCCCTTGCGTCCATAACA
MUC2 reverse primer			AGGAGCAGTGTCCGTCAAAG
SI forward primer			TCCAGCTACTACTCGTGTGAC
SI reverse primer			CCCTCTGTTGGGAATTGTTCTG
SPIB forward primer			CAGCAGCCGCTTTTAGCCAC
SPIB reverse primer			GCATATGCCGGGGGAACC