

Materials List for:

Clonal Analysis of Embryonic Hematopoietic Stem Cell Precursors Using Single Cell Index Sorting Combined with Endothelial Cell Niche Co-culture

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Materials

Name	Company	Catalog Number	Comments
AGM-EC culture media			
Materials for culture of endothelial cells			
TrypLE Express	Gibco	12605-028	Use to dissociate AGM-EC monolayers
Gelatin 0.1% in water	StemCell Technologies	7903	Use to adhere AGM-EC monolayers to plastic
96-well tissue culture plates	Corning	3599	Use to co-culture AGM-EC monolayers and index sorted clones
Dulbecco's Phosphate Buffered Saline (PBS)	Gibco	14190-144	Use for dissection and FACS staining
500 ml filter bottles	Fisher Scientific	9741202	Use to sterile filter Endothelial media
AGM-EC culture media (500 mls)			
Iscove's Modified Dulbecco's Medium (IMDM)	Gibco	12440-053	400 mls
Hyclone Fetal Bovine Serum	Fisher Scientific	SH30088.03	100 mls
Penicillin Streptomycin	Gibco	15140-122	5 mls
Heparin	Sigma	H3149	50 mg dissolved in 10 mls media
L-glutamine (200 mM)	StemCell Technologies	7100	5 mls
Endothelial Mitogen (ECGS)	Alfa Aesar	J64516 (BT-203)	Add 10 mls media to dissolve and add
Materials for AGM index sort			
Collagenase (0.25%)	StemCell Technologies	7902	Use for dissociation of embryonic tissues
3ml syringe	BD Biosciences	309657	Use to sterile filter antibody solutions for FACS
0.22 µm syringe-driven filter	Millipore	SLGP033RS	Use to sterile filter antibody solutions for FACS
5 ml polystyrene tube with cell-strainer cap	Corning	352235	Use to remove cell clumps prior to FACS
DAPI (prepared as 1 mg/ml stock in H ₂ O)	Millipore	268298	Use to exclude dead cells from sort
anti-mouse CD16/CD32 (FcR block)	BD Biosciences	553141	Use to block non-specific staining to Fc receptors
Antibodies for AGM index sort			
Anti-mouse CD144 PE-Cyanine7	eBioscience	25-1441-82	Staining

Rat IgG1 kappa Isotype Control, PE-Cyanine7	eBioscience	25-4301-81	Isotype control for CD144 PE-Cyanine7
Anti-mouse CD201 (EPCR) PerCP-eFluor710	eBioscience	46-2012-80	Staining
Rat IgG2b kappa Isotype Control, PerCP-eFluor710	eBioscience	46-4031-80	Isotype control for CD201 PerCP-eFluor710
Anti-mouse CD41 PE	BD Biosciences	558040	Staining
Rat IgG1 kappa Isotype Control, PE	BD Biosciences	553925	Isotype control for CD41 PE
Anti-mouse CD45 FITC	eBioscience	11-0451-85	Staining
Rat IgG2b kappa Isotype Control, FITC	eBioscience	11-4031-81	Isotype control for CD45 FITC
AGM-serum free media (10mls)			
X-Vivo 20	Lonza	04-448Q	10 mls
recombinant murine stem cell factor (SCF)	Peprtech	250-03	10 ml (100 mg/ml stock) Final concentration 100 ng/ml
recombinant human FLT3 Ligand (FLT3L)	Peprtech	300-19	10 ml (100 mg/ml stock) Final concentration 100 ng/ml
recombinant human thrombopoietin (TPO)	Peprtech	300-18	2 ml (100 mg/ml stock) Final concentration 20 ng/ml
recombinant murine interleukin-3 (IL3)	Peprtech	213-13	2 ml (100 mg/ml stock) Final concentration 20 ng/ml
Materials for Staining co-cultured cells			
96 well plate, V-bottom	Corning	3894	Use for FACS analysis
Antibodies for analysis of Index sorted clones co-cultured with endothelial cells			
Anti-mouse CD45 PerCP-Cyanine5.5	eBioscience	45-0451-82	Staining
Rat IgG2b kappa Isotype Control, PE-Cyanine5.5	eBioscience	35-4031-80	Isotype control for CD45 PerCP-Cyanine5.5
Anti-mouse CD201 (EPCR) PE	eBioscience	12-2012-82	Staining
Rat IgG2b kappa Isotype Control, PE	eBioscience	12-4031-81	Isotype control for CD201 PE
Anti-mouse Ly-6A/E (Sca-1) APC	eBioscience	17-5981-83	Staining
Rat IgG2a kappa Isotype Control, APC	eBioscience	17-4321-81	Isotype control for Ly-6A/E APC
Anti-mouse F4/80 FITC	eBioscience	11-4801-81	Staining
Rat IgG2a kappa Isotype Control, FITC	eBioscience	11-4321-41	Isotype control for F4/80 FITC
Anti-mouse Ly-6G/C (Gr1) FITC	BD Biosciences	553127	Staining
Rat IgG2b kappa Isotype Control, FITC	BD Biosciences	556923	Isotype control for Ly-6G/C and CD3 FITC
Materials for tail vein injection for transplant			
1/2 ml insulin syringes with 29G 1/2" needles	BD Biosciences	309306	Use for tail vein injection for transplantation
Antibodies for Peripheral Blood analysis following translat			
Anti-mouse Ly-6G/C (Gr1) PerCP	Biolegend	108426	Staining
Rat IgG2b kappa Isotype Control, PerCP	Biolegend	400629	Isotype control for Ly-6G/C PerCP

Anti-mouse F4/80 PE	eBioscience	12-4801-82	Staining
Rat IgG2a kappa Isotype Control, PE	eBioscience	12-4321-80	Isotype control for F4/80 PE
Anti-mouse CD3 FITC	BD Biosciences	555274	Staining
Anti-mouse CD19 APC	BD Biosciences	550992	Staining
Rat IgG2a kappa Isotype Control, APC	BD Biosciences	553932	Isotype control for CD19 APC
Anti-mouse CD45.1 (A20) PE-Cyanine7	eBioscience	25-0453-82	Staining
Rat IgG2a kappa Isotype Control, PE-Cyanine7	eBioscience	25-4321-81	Isotype control for CD45.1 PE-Cyanine7
Anti-mouse CD45.2 (104) APC-eFluor780	eBioscience	47-0454-82	Staining
Rat IgG2a kappa Isotype Control, APC-eFluor780	eBioscience	47-4321-80	Isotype control for CD45.2 APC-eFluor780
Equipment			
BD FACSAria II with DIVA software	BD Biosciences		
BD FACSCanto II with plate reader	BD Biosciences		
Haemocytometer	Fisher Scientific	S17040	For counting cells
Multi-channel pipette	Fisher Scientific	14-559-417	For dispensing cells
FACS analysis software	FlowJo/BD Biosciences		https://www.flowjo.com/solutions/flowjo