

Materials List for:

Promoting 3-D Aggregation of FACS Purified Thymic Epithelial Cells with EAK 16-II/EAKIIH6 Self-assembling Hydrogel

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Materials

Name	Company	Catalog Number	Comments
TEC Isolation			
70% Ethanol	Decon Laboratories	2701(1 Gallon)	Ethanol 200 Proof, deionized water
Dissecting scissors, straight	Fine Science Tools, Inc.	91460-11	
Graefe forceps, straight	Fine Science Tools, Inc.	11053-10	
Graefe forceps, curved	Fine Science Tools, Inc.	11052-10	
Washing solution			1x PBS, 0.1% BSA, 2 mM EDTA
1x PBS (Phosphate buffered saline)	Gibco	10010-023	
BSA (Bovine serum albumin)	Sigma	A1470-100G	
EDTA (ethylenediaminetetraacetic acid)	Invitrogen	15575-038	
Digestion solution			9 ml RPMI-1640, 0.025 mg/ml Liberase TM Research grade, 10 mM HEPES, 0.2 mg/ml DNaseI
RPMI-1640	Gibco	11879-020	
Liberase TM Research Grade	Roche	05 401 127 001	referred as "purified collagenase"
1 M HEPES	Lonza	17-737E	
Dnase I	Roche	10 104 159 001	
50 ml Centrifuge tube	Corning	430290	
60 mm tissue culture dish	Falcon	353002	
1/2 cc U-100 Insulin syringe 28 1/2 G	Becton Dickinson	329461	
5 ml Polystyrene round-bottom tube	Falcon	352058	
5 ml glass pipet	Fisher Healthcare	13-678-27E	Use for rinsing the thymic fragments. Thymic fragments tend to stick to the wall with plastic pipets.
MACSmix tube rotator	Miltenyi	130-090-753	
100 µm Cell strainer	Falcon	352360	
Density gradient medium: OptiPrep	Axis-Shield		
Cell Sorting			
5 ml Polypropylene round-bottom tube	Falcon	352063	
Anti-mouse CD16/CD32 (Fc Block)	BD Biosciences	553142	Use as undiluted, 2 µl per sample

Anti-mouse CD45-PerCPy5.5	eBioscience	45-0451-80	Use at 1:150, 10 µl per sample
Anti-mouse CD326 (EpCAM)-PE	eBioscience	12-5791-82	Use at 1:100, 10 µl per sample
BD Influx	BD Biosciences		
Single cell analysis software	FlowJo		
EAK Gel Assembly			
Anti-His-Tag	AnaSpec	29673	"anti-His-Tag IgG"
Purified anti-mouse CD326 (EpCAM)	BioLegend	118202	"anti-EpCAM IgG"
Recombinant protein A/G	Pierce Biotechnology		
1.5 ml Safe-Lock Tubes, Biopur, Sterile	Fisher Healthcare	05-402-24B	referred as "1.5 ml microcentrifuge tube"
96-well, Tissue culture plate, Round-bottom with low evaporation lid	BD Falcon	353917	
Rocking platform: Nutator Mixer no.1105	BD Clay Adams		
10% sucrose	Sigma	S0389	Prepare with sterile distilled water
EAK16-II (AcNH-AEAEAKAKAEAEAKAK-CONH ₂)	American Peptide Company		custom synthesized, 10 mg/ml
EAKIIH6 (AcNH-AEAEAKAKAEAEAKAKHHHHH-CONH ₂)	American Peptide Company		custom synthesized, 7.5 mg/ml
Complete medium			RPMI-1640, 10% FBS, 1% Pen/Strep, 1% L-glutamine, 1% NEAA, 5 mM HEPES, 50 µM 2-Mercaptoethanol
RPMI-1640	Gibco	11879-020	
FBS (Fetal Bovine Serum)	Atlanta Biologicals	S11150	Heat inactivated before use
Pen/Strep	Gibco	15140-122	
L-glutamine 200 mM (100x)	Gibco	25030-081	
NEAA (non-essential amino acid) 100x	Gibco	11140-050	
1 M HEPES	BioWhittaker	17-737E	
2-Mercaptoethanol (100x)	Millipore	ES-007-E	
Platform shaker: The Belly Dancer	Stovall Life Sciences Inc.	model: USBDb0	