

Materials List for:

Generation of Human Regulatory T Cell Clones

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Materials

Name	Company	Catalog Number	Comments
0.22 µm Stericup, 500 mL	Millipore	5500	Media storage and preparation
100x Nonessential amino acids	Gibco	11140-050	Media component
15 mL conical centrifuge tubes (50/ bag, case of 500)	ThermoFisher Scientific	339650	
1M HEPES	Gibco	15630-080	Media component
25 ml Single Well Pipet Basin	Fischer Scientific	13-681-508	
50 mL Conical Centrifuge Tube (25/sleeve)	ThermoFisher Scientific	339652	
50x Penicillin Streptomycin Soln	Corning	Corning, 30-001-CI	Media component
CryoTube Vial Int Thread Round Btm Starfoot PP Screw Stopper Sterile PP 1.8 mL	Nalge Nunc	377267	
DMSO	Corning	25-950-CQC	
EasySep Human CD25 positive selection kit	Stemcell Technologies	18231	Alternatives are FACS or MACS column-based sorting
EasySep Human CD4+CD127low T cell Pre-Enrichment Kit	Stemcell Technologies	19231	Alternatives are FACS or MACS column-based sorting
EasySep Human CD4+CD127lowCD25+ Regulatory T Cell Isolation Kit (alternative to item 12)	Stemcell Technologies	18063	Alternatives are FACS or MACS column-based sorting
Ficoll	GE Healthcare	17-5442-03	PBMC purification from peripheral blood of leukapheresis products; density gradient medium
Human AB Serum (PHS-AB)	Valley Biomedical Inc	HP1022	Media component
LIVE/DEAD Fixable Blue Dead Cell Stain Kit, for UV excitation	Thermo Fischer	L-34962	Viability dye
Phytohemagglutinin-L (PHA-L)	Millipore/Sigma	11249738001	T cell stimulation
Recombinant IL-2 (e.g., PROLEUKIN [®])	Prometheus		T cell stimulation and maintenance/ Media component
RPMI 1640	Gibco	21870-076	Media component
Staining antibodies for flowcytometry (Treg phenotyping)	See "Comments"	See "Comments"	Staining antibodies are enlisted in: Nowatzky et al. (2019) PubMed PMID: 30584695; PubMed Central PMCID: PMC6497402. In case EasySep Human CD25 positive selection kit is used, stain with 2A3 or BC96 anti-CD25 antibody, e.g.: Brilliant Violet 421 anti-human CD25 Antibody (Biolegend; 302629)

TCR V β Repertoire Kit; IOtest Beta Mark	Beckman Coulter	PN IM3497	Vetting of expansions for monoclonality
Tissue Culture Plate, 96 Well, U-Bottom with Low Evaporation Lid	Corning	353077	