

Materials List for:

# Isolation and Activation of Murine Lymphocytes

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## Materials

Name	Company	Catalog Number	Comments
<b>Materials</b>			
RPMI 1640 (without L-Glutamine)	Gibco	31870025	
Fetal Bovine Serum			Heat inactivated
L-glutamine	Gibco	25030024	
Penicillin/Streptomycin	Gibco	15140114	
2-mercaptoethanol	Gibco	21985023	
Anti-CD43 magnetic microbeads	Miltenyi Biotec	130-049-801	Mix well prior use
Streptavidin microbeads	Miltenyi Biotec	130-048-101	Mix well prior use
Anti-Annexin V magnetic beads	Miltenyi Biotec	130-090-201	Mix well prior use
MACS LD	Miltenyi Biotec	130-042-901	
96-well U-bottom sterile culture plate	Greiner Bio-one	650180	
96-well F-bottom sterile culture plate	Greiner Bio-one	655180	
100 µm cell strainer mesh			To sterilize using UV radiation prior use
0.2 µm sterile disposable filter units	Nalgene	567-0020	Can be substituted with any sterile filter device
CellTrace Violet	Invitrogen	C34557	CTV for short; alternative to CFSE
CellTrace Yellow	Invitrogen	C34567	CTY for short; alternative to CFSE
CellTrace Far Red	Invitrogen	C34564	CTFR for short; alternative to CFSE
Cell Proliferation Dye eFluor 670	eBioscience	65-0840	CPD670 for short; alternative to CFSE
PKH26	Sigma Aldrich	PKH26GL	PKH26, alternative to CFSE
<b>Chemicals</b>			
Dextrose	Sigma Aldrich	G7021	
Potassium phosphate monobasic	Sigma Aldrich	P5655	
Sodium phosphate dibasic	Sigma Aldrich	S5136	
Phenol Red	Sigma Aldrich	P0290	
Calcium chloride dihydrate	Sigma Aldrich	C7902	
Potassium chloride	Sigma Aldrich	P5405	
Sodium chloride	Merck Millipore	S7653	Can use from other sources
Magnesium chloride hexahydrate	Sigma Aldrich	M2393	
Magnesium sulfate	Sigma Aldrich	M2643	
Ammonium chloride	Sigma Aldrich	A9434	

Tris-base			
Dimethyl Sulfoxide	Sigma Aldrich	D8418	
(5-(and 6-) carboxyfluorescein diacetate succinimidyl ester (CFSE)	Molecular Probes	C-1157	Reconstitute in DMSO
Phorbol 12,13-dibutyrate (PBDU, Phorbol ester)	Sigma Aldrich	P1269	
A23187 (Calcium ionophore)	Sigma Aldrich	C7522	
<b>Name</b>	<b>Company</b>	<b>Catalog Number</b>	<b>Comments</b>
<b>Antibodies and recombinant protein</b>			
CD11b biotin (clone m1/70)	Biolegend	101204	T cell depletion cocktail
CD11c biotin (clone N418)	Biolegend	117304	T cell depletion cocktail
Gr-1 biotin (clone RB6-8C5)	Biolegend	108404	T cell depletion cocktail
Ter119 biotin (clone Ter119)	Biolegend	116204	T cell depletion cocktail
TCR- $\gamma\delta$ biotin (clone GL-3)	Biolegend	118103	T cell depletion cocktail
CD19 biotin (clone 6D5)	Biolegend	115504	T cell depletion cocktail
B220 biotin (clone RA3-6B2)	Biolegend	103204	T cell depletion cocktail
CD49b biotin (clone DX5)	Biolegend	108904	T cell depletion cocktail
CD4 biotin (clone GK1.5)	Biolegend	100404	T cell depletion cocktail
CD8 biotin (clone 53-6.7)	Biolegend	100704	T cell depletion cocktail
F(ab') <sub>2</sub> goat anti-mouse IgM (plate coated)	Jackson ImmunoResearch	115-006-075	50 $\mu$ l/well for coating (96-well)
Anti-mouse CD40 mAb (plate coated)	Pharmingen	553722	50 $\mu$ l/well for coating (96-well)
Recombinant IL-4	ProSpec	Cyt-282	
LPS from E. coli Serotype 055:B5	Sigma Aldrich	L-4005	
Anti-CD3 (clone clone OKT3) (plate coated)	eBioscience	16-0037-85	50 $\mu$ l/well for coating (96-well)
Anti-CD28 (clone clone 37.51) (plate coated)	eBioscience	16-0281-85	50 $\mu$ l/well for coating (96-well)
Recombinant IL-2	ProSpec	Cyt-370	
Albumin from chicken egg white, Ovalbumin	Sigma Aldrich	A7641	