

Materials List for:

Mouse Naïve CD4⁺ T Cell Isolation and *In vitro* Differentiation into T Cell Subsets

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Materials

Name	Company	Catalog Number	Comments
Complete RPMI:			Warm in a 37 °C water bath before use
RPMI 1640 Media	Life Technologies	11875119	
10 % FBS	Life Technologies	26140-079	
1000X 2-mercaptoethanol	Life Technologies	21985023	
100X Pen/Strep	Life Technologies	15140122	
100X L-glutamine	Life Technologies	25030081	
120 micron nylon mesh	Amazon	CMN-0120-10YD	Cut into 2 cm ² squares and autoclave
Alternative: 100 micron cell strainers	Fisher	08-771-19	Alternative to cutting nylon mesh
autoMACS running buffer	Miltenyi	130-091-221	Warm in a 37 °C water bath before use
autoMACS rinsing solution	Miltenyi	130-091-222	Warm in a 37 °C water bath before use
CD4 beads	Miltenyi	130-049-201	
ACK lysis buffer	Life Technologies	A10492-01	
Cytokines:			
Human (h) IL-2	Peprtech	200-02	
Recombinant mouse (rm) IL-4	Peprtech	214-14	
rmIL-6	R & D Systems	406-ML-025	
rmIL-12	Peprtech	210-12	
hTGFb	R & D Systems	240-B-010	
Antibodies:			
2C11 (anti-CD3)	BioXcell	BE0001-1	
37.51 (anti-CD28)	BioXcell	BE0015-1	
11B11 (anti-IL-4)	BioXcell	BE0045	
XMG1.2 (anti-IFNγ)	BioXcell	BE0055	
anti-CD62L-FITC	BioLegend	104406	Use at 1:100
anti-CD25-PE	BioLegend	102008	Use at 1:400
anti-CD4-PerCP	BioLegend	100434	Use at 1:1000
anti-CD44-APC	BioLegend	103012	Use at 1:500
Phorbol 12-myristate 13 acetate (PMA)	Sigma-Aldrich	P-8139	Prepare a stock at 0.1 mg/ml in DMSO and freeze aliquots at -20 °C

Ionomycin	Sigma-Aldrich	I-0634	Prepare a stock at 0.5 mg/ml in DMSO and freeze aliquots at -20 °C
Brefeldin A	eBioscience	00-4506-51	Use at 1:1000