

Materials List for:

An Efficient and High Yield Method for Isolation of Mouse Dendritic Cell Subsets

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Materials

Name	Company	Catalog Number	Comments
0.05% Trypsin-EDTA	Life Technologies, Gibco	25300-054	
Isoflurane	Sigma-Aldrich	CDS019936-250MG	
Collagenase D	Roche Diagnostics	11088858001	
DNase I (dry powder)	QIAGEN	79254	
200 proof ethanol	Thermo Fisher Scientific	9-6705-004-220	Used to prepare 70% ethanol
RBC lysis buffer	Sigma-Aldrich	R7757	
RPML-1640 medium with L-glutamine	Life Technologies, Gibco	11875-119	
DMEM medium with L-glutamine	Life Technologies, Gibco	11995-073	
200 mM L-glutamine	Life Technologies	25030081	
MEM non-essential amino acids	Life Technologies, Gibco	11140-050	
MEM essential amino acids	Life Technologies	11130-051	
β-mercaptoethanol	Life Technologies, Invitrogen	21985-023	
Sodium pyruvate	Life Technologies	11360-070	
HEPES	Life Technologies, Invitrogen	15630	
Phosphate buffered saline (PBS Ca ²⁺ and Mg ²⁺ free pH 7.2)	Life Technologies, Invitrogen	20012-050	
Dulbecco's PBS (DPBS with Ca ²⁺ and Mg ²⁺)	Life Technologies, Gibco	14040-182	
0.5 M Ethylenediaminetetraacetate (EDTA) solution	Life Technologies	15575-020	
Bovine serum albumin (BSA)	Sigma-Aldrich	A2153	
Fetal calf serum	Atlanta Biologicals	S11050	
Penicillin/streptomycin	Life Technologies, Invitrogen	15140-163	
Trypan blue (dry powder)	Sigma-Aldrich	T6146-5G	Prepare 0.08% with PBS
Plasmacytoid Dendritic Cell Isolation Kit II, mouse	Miltenyi Biotech	130-092-786	
Magnetic beads conjugated with anti-mouse CD11c	Miltenyi Biotech	130-152-001	
CD8α ^{Pos} mouse DC isolation kit	Miltenyi Biotech	130-091-169	
Fc-gamma receptor blocking antibody (Clone 2.4G2)	BD Biosciences	553141	
Anti-mouse CD11c-FITC	BD Biosciences	553801	
Anti-mouse CD8α-PerCP	BD Biosciences	553036	
Anti-mouse B220-PE	BD Biosciences	553090	

1 ml syringes	BD	26048	
23 G1 needle	BD	305145	
100 mm Petri dishes	Thermo Fisher Scientific	875712	
Surgical instruments	Kent Scientific	INSMOUSEKIT	
Cell strainer (70 µm)	BD	352350	
Large Petri plates	Thermo Fisher Scientific	FB0875712	
Vacuum filtration system (500 ml (0.22 µm))	Corning	431097	
LS columns	Miltenyi	130-042-401	
Magnetic stand MACS separator	Miltenyi Biotec	130-042-302	
Wide-bore 200 µl pipette tips	PerkinElmer	111623	
Corning ultra-low attachment 96-well plates	Corning	CLS3474-24EA	
6-8 week old female C57BL/6 mice	Jackson Research Laboratories, Jax		
Murine B16.F1t3L melanoma cell line			described by Mach <i>et al.</i> , 2000
Serum free DMEM and RPMI media 500 ml DMEM with L-glutamine, or 500 ml RPMI-1640 with L-glutamine 5 ml MEM nonessential amino acids (100x, 10 mM) 5 ml HEPES Buffer (1 M) 5 ml L-glutamine (200 mM) 0.5 ml 2-mercaptoethanol (5.5 x 10 ⁻² M)			Mix all the ingredients in a biosafety cabinet with either DMEM or RPMI media depending on your need. Filter sterilize the media by passing through a 0.22 µm vacuum filtration system.
Complete RPMI and DMEM media			Add 50 ml of heat inactivated fetal calf serum to serum free RPMI or DMEM media to obtain complete media.
MACS buffer			Add 2 ml of 0.5 M EDTA and 10 ml of heat inactivated fetal calf serum to 500 ml of Phosphate-buffered saline (PBS, Ca ²⁺ and Mg ²⁺ Free, pH 7.2) and 2 µg/ml 2.4G2 (Fc-Block antibody).
FACS staining buffer			Dissolve sodium azide to 0.05% (0.25 g per 500 ml) in MACS buffer to obtain FACS staining buffer
10x Collagenase D and DNase 1 stock solution Dissolve 1 g of collagenase D and 0.2 ml of DNase 1 stock (1 mg/ml, 100x) in 20 ml of PBS containing Ca ²⁺ and Mg ²⁺ to obtain a solution of approximately 1,000-2,000 Units of collagenase activity per ml.			This 10x stock solution can be prepared ahead of time and stored at -20 °C for several weeks. Dilute 1 ml of this with 9 ml of serum free RPMI immediately before use.