

Materials List for:

Isolation of Infiltrating Leukocytes from Mouse Skin Using Enzymatic Digest and Gradient Separation

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Materials

Name	Company	Catalog Number	Comments
HBSS with phenol red, without calcium, without magnesium, liquid	Sigma Aldrich	55021C	Keep sterile until day of usage.
HEPES, ≥99.5% (titration)	Sigma Aldrich	H3375	
EDTA disodium salt solution	Sigma Aldrich	E7889	Keep sterile until day of usage.
Fetal Bovine Serum, USDA, Heat Inactivated, Premium Select	MidSci	S01520HI	Keep sterile until day of usage.
Percoll, pH 8.5-9.5 (25°C)	Sigma Aldrich	P1644	Keep sterile. Percoll needs to be made isotonic with sterile 10X PBS prior to use.
Trimmer Combo Kit	Kent Scientific	CL9990-1201	Use the larger trimmer for shaving the flank and back.
4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one	Sigma Aldrich	E0753-10G	Dissolve in 100% EtOH at 50°C for 15 minutes on a rotating plate.
Purified anti-mouse CD16/CD32 (2.4G2)	Tonbo Biosciences	70-0161	Antibody used to block non-specific antibody binding during antibody staining for flow cytometry
anti-CD3ε-BV650 (145-2C11)	Becton Dickinson	564378	Antibody used to stain cells for flow cytometry
anti-CD4-APC-eFluor780 (RM4-5)	eBioscience	47-0042-80	Antibody used to stain cells for flow cytometry
anti-CD8α-BV785 (53-6.7)	BioLegend	100749	Antibody used to stain cells for flow cytometry
anti-CD11b-eFluor450 (M1/70)	eBioscience	48-0112-80	Antibody used to stain cells for flow cytometry
anti-CD11c-eFluor450 (N418)	eBioscience	48-0114-80	Antibody used to stain cells for flow cytometry
anti-CD44-BV711 (IM7)	Becton Dickinson	563971	Antibody used to stain cells for flow cytometry
anti-CD45-FITC (30-F11)	Tonbo Biosciences	35-0451-U025	Antibody used to stain cells for flow cytometry
anti-CD45R-eFluor450 (RA3-6B2)	eBioscience	48-0452-80	Antibody used to stain cells for flow cytometry
anti-CD62L-PerCP-Cy5.5 (MEL-14)	BioLegend	104431	Antibody used to stain cells for flow cytometry
anti-Ly-6G/Ly-6C (Gr-1)-PE (RB6-8C5)	BioLegend	108407	Antibody used to stain cells for flow cytometry
Ghost Dye-BV510	Tonbo Biosciences	13-0870-T100	Viability dye for flow cytometry
LSR Fortessa	Becton Dickinson	N/A	Cell analyzer (18 parameters)
Collagenase D from <i>Clostridium histolyticum</i>	Roche Applied Science	11088858001	Aliquot lyophilized enzyme at 5 mg/ml in HBSS with phenol

			red, without calcium, and without magnesium into 1 mL aliquots. Store immediately at -20°C for up to six months.
ND4 Swiss female mice	Harlan	0-32	8-12 weeks old; conventionally housed with free access to food and water and used according to Macalester College's IACUC guidelines.