

Materials List for:

# Isolation and Characterization of Neutrophils with Anti-Tumor Properties

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## Materials

Name	Company	Catalog Number	Comments
CELL LINES			
Mouse 4T1 breast carcinoma cells	ADCC	CRL-2539	Growth medium: DMEM + 10 % heat-inactivated FBS
PLASTIC WARES AND EQUIPMENTS			
24-well Tissue Culture Plate	Falcon	353047	Sterile
100 mm Tissue Culture Plate	Corning	430167	Sterile
25 cm <sup>2</sup> Tissue Culture Flask	Nunc	156340	Sterile
90 mm Bacterial Grade Culture Dish	Miniplast, Ein Shemer, Israel	20090-01-017	Sterile
15 ml Sterile Conical Centrifuge Tube	Miniplast, Ein Shemer, Israel	835015-40-111	Sterile
50 ml Sterile Conical Centrifuge Tube	Miniplast, Ein Shemer, Israel	835050-21-111	Sterile
Falcon 12 x 75 mm Round-Bottom Polystyrene Tube	Becton Dickinson	352058	Sterile
Millicell 24 Migration Plate with a pore size of 5µm	Merck Millipore	PSMT010R1	Sterile
White 96-Flat-Bottom Well Plate	Costar	3917	Sterile
Cell Strainer (40 mm)	BD Falcon	352340	Sterile
20G x 1.5" Needle	BD Microlance 3	301300	Sterile
23G x 1" Needle	BD Microlance 4	300800	Sterile
25G x 5/8" Needle	BD Microlance 5	300600	Sterile
0.3 ml Syringe with a 30G x 8mm Needle	BD Micro-Fine Plus Demi	320829	Sterile
9 mm Clips	BD, AutoClip	427631	Sterile
EasySep Magnet	STEMCELL Technologies	18000	
MACS LS Separation Column	Miltenyi Biotech	130-042-201	Sterile
MidiMACS Separator Magnet	Miltenyi Biotech	130-042-302	
MACS MultiStand	Miltenyi Biotech	130-042-303	
Microscope Glass Slide	Menzel-Gläser Superfrost Plus Thermo	J1800AMNZ	
Orbital Shaker	Sky line, ELMI	S-3.02.10L	
Plate Reader	TECAN	InfiniteF200Pro	
POWDER			
Bovine serum albumin (BSA), fraction V	Sigma	A7906	

Bromodeoxyuridine (BrdU)	BD Pharmingen	550891	Sterile
CFSE (5-(and 6-)-Carboxyfluorescein diacetate, succinimidyl ester)	Molecular Probes	C1157	
Dextran T500	Sigma	31392	
Heparin sodium salt from porcine intestinal mucosa	Sigma	H3149	
Sodium azide (NaN <sub>3</sub> )	Sigma	S8032	Highly toxic, handle with care
Thioglycollate powder	Difco	225650	
Zyosan A	Sigma	Z4250	
MEDIA AND SUPPLEMENTS			
Dulbecco's modified Eagle medium (DMEM)	Sigma	D5796	Sterile
Opti-MEM® I reduced serum medium	Life Technologies	31985062	Sterile
Roswell Park Memorial Institute (RPMI)-1640 medium	Sigma	R8758	Sterile
Foetal bovine serum (FBS), heat-inactivated	Sigma	F9665	Sterile
L-Glutamine	Biological Industries, Beth HaEmek, Israel	03-020-1A	Sterile
Sodium pyruvate	Biological Industries, Beth HaEmek, Israel	03-042-1B	Sterile
Penicillin Streptomycin x 1000 solution	Biological Industries, Beth HaEmek, Israel	03-031-5	Sterile
Phosphate buffered saline (PBS) without Mg <sup>2+</sup> and Ca <sup>2+</sup>	Biological Industries, Beth HaEmek, Israel	02-023-1	Sterile
PBS x 10 without Ca <sup>2+</sup> and Mg <sup>2+</sup>	Biological Industries, Beth HaEmek, Israel	02-023-5A	Sterile
HPLC grade water	J.T. Baker	4218-03	Autoclave
SOLUTIONS			
ACK – Ammonium-Chloride-Potassium	Life Technologies	A10492-01	
Bromodeoxyuridine (BrdU) solution (10 mg/ml) in PBS			Dissolve 10 mg of BrdU in 1 ml PBS and sterile filter.
CFSE, 5 mM in DMSO			Dissolve 2.8 mg of CFSE in 1 ml DMSO. Divide into 10 ml aliquots in sterile 200 ml tubes and store in the dark at -20°C.
Eosin Y solution	Sigma	HT110-2-32	
Hanks' balanced salt solution	Biological Industries, Beth HaEmek, Israel	02-016-1A	Sterile
Heparin, 20 mg/ml in PBS			Dissolve 100 mg Heparin in 5 ml sterile PBS, and sterile filter through a 0.2 mm filter.
Histopaque-1119	Sigma	11191	Sterile filter through a 0.2 mm filter.
Histopaque-1077	Sigma	10771	Sterile filter through a 0.2 mm filter.
Luciferase cell culture lysis buffer x5	Promega	E153A	Dilute 1:5 in sterile water just before use.
Luciferase assay solution	Promega	E1501	Contains luciferase assay substrate powder (E151A) and luciferase assay buffer (E152A)
Mayer's Hematoxylin solution	Sigma	MHS-32	

PBS + 0.5% BSA			Dissolve 2.5 g BSA in 500 ml PBS, and sterile filter through a 0.2 mm filter.
PBS + 1% BSA			Dissolve 1g BSA in 100 ml PBS, and sterile filter through a 0.2 mm filter.
5x PBS with 2.5% BSA			Dissolve 12.5g BSA in a mixture of 250 ml sterile HPLC-grade water and 250 ml PBSx10, and sterile filter through a 0.2 mm filter.
PBS containing 0.5% BSA and 2 mM EDTA			Dissolve 250 mg BSA in 50 ml sterile PBS and add 200 ml of 0.5M EDTA pH 8.0, sterile filter through a 0.2 mm filter.
FACS buffer (PBS containing 0.5% BSA, 2 mM EDTA and 0.02% NaN <sub>3</sub> )			Dissolve 250 mg BSA in 50 ml sterile PBS and add 200 ml of 0.5M EDTA pH 8.0 and 500 ml of 2% NaN <sub>3</sub> , sterile filter through a 0.2 mm filter.
Saline (0.9% NaCl)			Dissolve 9 g NaCl in 1,000 ml ddw, autoclave
0.2% NaCl solution			Dissolve 2 g NaCl in 1,000 ml ddw, autoclave
1.6% NaCl solution			Dissolve 16 g NaCl in 1,000 ml ddw, autoclave
2 % Sodium azide			Dissolve 1g sodium azide in 50 ml sterile ddw, keep at 4°C. Highly toxic.
3% Thioglycollate solution			Dissolve 3 g of thioglycollate powder in 100 ml ddw. Boil until solution becomes yellow and autoclave.
Trypan blue solution (0.4%)	Sigma	T8154	Dilute 1:10 in PBS to get a 0.04% solution.
Trypsin solution B	Biological Industries, Beth HaEmek, Israel	03-046-1	Sterile
1 mg/ml Zymosan A			Resuspend 1 mg Zymosan A in 1 ml sterile PBS in an Eppendorf tube. Vortex vigorously and incubate the tube at 37 °C for 30 min. Do not autoclave. Prepare the solution freshly before use.
KITS			
EasySep PE selection kit	STEMCELL Technologies	18557	
EasySep PE selection cocktail	STEMCELL Technologies	18151	
the EasySep magnetic nanoparticles	STEMCELL Technologies	18150	
Anti-Ly6G mouse MicroBead Kit	Miltenyi Biotec	130-092-332	
EasySep Mouse Neutrophil Enrichment Kit	STEMCELL Technologies	19762	
EasySep Human Neutrophil Enrichment Kit	STEMCELL Technologies	19257	
FITC BrdU flow kit	BD Pharmingen	559619	
MACS Neutrophil isolation kit	Miltenyi Biotec	130-097-658	
Phagocytosis Assay Kit	Cayman Chemical Company	500290	

ANTIBODIES			
FcR blocking antibody	Biologend	101302	
Purified rat anti-Ly6G antibody	BD Pharmingen	551459	Clone 1A8
PE-conjugated rat anti-mouse Ly6G antibody	Biologend	127608	Clone 1A8
FITC-conjugated rat anti-mouse Ly6G	BD Pharmingen	551460	Clone 1A8
PerCP-Cy5.5 rat anti-mouse Ly6G	TONBO Biosciences	65-1276	Clone 1A8
violetFluor 450-conjugated rat anti-mouse Ly6G	TONBO Biosciences	75-1276	Clone 1A8
FITC-conjugated rat anti-mouse CD11b	BD Pharmingen	553310	Clone M1/70
FITC-conjugated rat anti-mouse Ly-6G and Ly-6C (GR-1)	BD Pharmingen	553127	Clone RB6-8C5
PE-conjugated rat anti-mouse CD45	BD Pharmingen	553081	Clone 30-F11
FITC-conjugated rat anti-mouse F4/80	Abcam	ab60343	Clone BM8
FITC-conjugated mouse anti-human CD66b	Biologend	305103	Clone G10F5
Purified rat isotype control antibody (IgG <sub>2a</sub> , k)	BD Pharmingen	553927	Clone R35-95
LEAF purified Armenian hamster anti-mouse CD3e antibody	BioLegend	100314	Clone 145-2C11