

Materials List for:

An Optimized Protocol to Analyze Glycolysis and Mitochondrial Respiration in Lymphocytes

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Materials

Name	Company	Catalog Number	Comments
PBS (pH 7.2)	Gibco-ThermoFisher	20012-050	To make MACS buffer
Bovine Serum Albumin BSA	Sigma-Aldrich	A3803	To make MACS buffer
0.5 M EDTA, pH 8	Quality Biological	10128-446	To make MACS buffer
autoMACS rinsing solution	Miltenyi Biotec	130-091-222	Instead of PBS + EDTA to make MS buffer. Also used in autoMACS Pro Separator.
RPMI-1640	Gibco-ThermoFisher	11875-093	Contains phenol red and L-glutamine
Fetal Bovine Serum	Gibco-ThermoFisher	10437-028	For heat-inactivation, thaw frozen stock bottle in a 37 °C water bath and then inactivate at 56 °C for 30 min. Aliquot heat-inactivated serum for storage (e.g., in 50 ml conical tubes) and freeze at -20 °C until needed.
Penicillin-Streptomycin (Pen Strep)	Gibco-ThermoFisher	15140-122	Combine Pen Strep with L-Glut 1:1 (if making 500 ml media, make a total of 12 ml Pen Strep/L-Glut); keep aliquots at -20 °C until ready to make media.
L-Glutamine 200 mM (L-Glut)	Gibco-ThermoFisher	25030-081	Component of RF10 and stress test media
Sodium pyruvate 100 mM	Gibco-ThermoFisher	11360-070	Component of RF10 and stress test media
HEPES 1 M	Gibco-ThermoFisher	15630-080	Component of RF10
MEM Non-Essential Amino Acids Solution (100x)	Gibco-ThermoFisher	11140-050	Component of RF10
2-Mercaptoethanol (55 mM)	Gibco-ThermoFisher	21985-023	Component of RF10
Falcon 70 µm cell strainer	Falcon-Fischer Scientific	87712	Used in cell isolation
Monoject 3 ml Syringe, Luer-Lock Tip	Covidien	8881513934	Used in cell isolation
Falcon 15 ml Conical Centrifuge Tubes	Falcon-Fischer Scientific	14-959-53A	Used in cell isolation
Falcon 50 ml Conical Centrifuge Tubes	Falcon-Fischer Scientific	14-432-22	Used in cell isolation
ACK Lysing Buffer	Lonza	10-548E	Used in cell isolation
CellTrics 30 µm cell filter, sterile, single-packed	Partec CellTrics	04-004-2326	Used in cell isolation

B Cell Isolation Kit, mouse	Miltenyi Biotec	130-090-862	Used in cell isolation
Naïve CD4 ⁺ T cell isolation kit, mouse	Miltenyi Biotec	130-104-453	Used in cell isolation
LS Column	Miltenyi Biotec	130-042-401	Used in cell isolation
MidiMACS separator	Miltenyi Biotec	130-042-302	Magnet for separation
MACS MultiStand	Miltenyi Biotec	130-042-303	Holder for magnets
autoMACS Rinsing Solution		130-091-222	Rinsing solution for autoMACS Pro Separator
autoMACS Pro Separator Instrument	Miltenyi Biotec	N/A	
2-Deoxy-D-glucose (2-DG)	Sigma-Aldrich	D8375-5G	
Cell-Tak	Corning	354240	Cell adhesive. Take care to note concentration, as each lot is different (package is 1 mg).
Oligomycin	Sigma-Aldrich	75351	
2,4-Dinitrophenol (2,4-DNP)	Sigma-Aldrich	D198501	
Antimycin A	Sigma-Aldrich	A8674	
Rotenone	Sigma-Aldrich	R8875	
Glucose	Sigma-Aldrich	G8270	
Halt Protease Inhibitor Cocktail	ThermoFisher Scientific	78429	Supplied as 100x cocktail, combine with RIPA to form 1x solution for lysis.
RIPA Buffer	Boston BioProducts	BP-115	
Pierce BCA Protein Assay Kit	ThermoFisher Scientific	23225	Follow manufacturer's instructions
Seahorse XFe96 FluxPak	Seahorse Bioscience	101085-004	Includes assay plates, cartridges, loading guides for transferring compounds to the assay cartridge, and calibrant solution.
Seahorse XF Base Medium	Seahorse Bioscience	102353-100	Used to prepare stress test media
Seahorse XFe96 Extracellular Flux Analyzer	Seahorse Bioscience		
Stericup Sterile Vacuum Filter Units, 0.22 µm	EMD Millipore-Fisher Scientific	SCGPU10RE	Used to sterile filter media.
Sodium bicarbonate	Sigma-Aldrich	487031	Dissolved to 0.1 M and used to dilute Cell-Tak.