

Materials List for:

# Metabolic Characterization of Polarized M1 and M2 Bone Marrow-derived Macrophages Using Real-time Extracellular Flux Analysis

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URL: <https://www.jove.com/video/53424>

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## Materials

Name	Company	Catalog Number	Comments
23G and 25G needles	Becton Dickinson	#300800 and #300600	To flushed bone marrow
10 ml syringes	Becton Dickinson	#307736	To flushed bone marrow
Petri dishes	Greiner	#639161	To culture bone marrow cells
CyQUANT Cell Proliferation Assay kit	Molecular Probes	#C7026	For cell quantification at a later time point (works only for adherent cells)
CyQUANT Direct Cell Proliferation Assay kit	Molecular Probes	#35011	For cell quantification immediately after assay (non-adherent cells)
XF <sup>e</sup> 96 cell culture microplate	Seahorse Bioscience	#101085-004	96 well plate in which cells are cultured and in which assay is done
recombinant mouse interleukin-4 (IL-4)	Peprotech	#214-14-B	Used to induced alternative macrophage activation
lipopolysaccharide (LPS)	Sigma	#L2637	Pro-inflammatory stimulus
Seahorse Sensor Cartridge	Seahorse Bioscience	#102416-100	Contains the probes for pH and O2 measurement
Seahorse XF Calibrant	Seahorse Bioscience	#100840-000	Needed to hydrate the probes overnight
XF base medium	Seahorse Bioscience	#102353-100	Buffer-free medium that allows efficient measurement of pH changes
L-glutamine	Life Technologies	#25030-081	
D-(+)-Glucose	Sigma	#G8769	Fuels glycolysis once added to the cells
oligomycin A	Sigma	#75351	Inhibits mitochondrial ATP synthase
FCCP( Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone)	Sigma	#C2920	Uncouples mitochondrial respiration
100 mM Sodium Pyruvate solution	Lonza	#BE13-115E	Allows maximal mitochondrial respiration without the need for glycolysis
Antimycin A	Sigma	#BE13-115EA8674	Inhibits complex III of the mitochondria
Rotenone	Sigma	#BE13-115EA8674R8875	Inhibits complex I of the mitochondria
Casy Cell Counter	Roche Diagnostics	#05 651 697 001	Instrument to count cells
anti-CD16/CD32	eBioscience	#14-0161	Fc receptor block flow cytometry
anti-F4/80-APC-eFluor780	eBioscience	#47-4801	Antibody to stain macrophages
anti-CD11b-FITC	eBioscience	#11-0112	Antibody to stain macrophages