

# High-Resolution Fluorespirometry to Assess Dynamic Changes in Mitochondrial Membrane Potential in Human Immune Cells

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## Materials

Name	Company	Catalog Number	Comments
Adenosine Diphosphate	Sigma-Aldrich	A5285	Fluorespirometry
Antimycin A	Sigma-Aldrich	A8674	Fluorespirometry
Bovine Serum Albumin (BSA)	Sigma-Aldrich	A6003	Mir05 buffer
Bovine Serum Albumin	Sigma-Aldrich	A6003	Cell isolation
Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone	Sigma-Aldrich	C2920	Fluorespirometry
Cell strainers	Fisher Scientific	22-363-548	Isolation of T-cells from mouse spleen protocol
CD14 Microbeads, human	Miltenyi Biotec	130-050-201	Cell isolation
CD3 Microbeads, human	Miltenyi Biotec	130-050-101	Cell isolation
DatLab	Oroboros	Version 8	
Digitonin	Sigma-Aldrich	D141	Fluorespirometry
D-Sucrose	Sigma-Aldrich	84097	Mir05 buffer
Ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA)	Sigma-Aldrich	E4378	Mir05 buffer
Filter Set AmR	Oroboros	44321-01	
HBSS (10x)	Gibco	12060-040	
HEPES sodium salt	Sigma-Aldrich	H7523	Mir05 buffer
Histopaque 1077	Sigma-Aldrich	10771	Cell isolation
K <sub>2</sub> EDTA blood collection tubes	BD Vacutainer	366643	Cell isolation
Lactobionic acid	Sigma-Aldrich	153516	Mir05 buffer
L-Glutamic acid	Sigma-Aldrich	G1626	Fluorespirometry
L-Malic Acid	Sigma-Aldrich	M1000	Fluorespirometry
LS Columns	Miltenyi Biotec	130-042-401	Cell isolation
Magnesium Chloride (MgCl <sub>2</sub> )	Sigma-Aldrich	M9272	Mir05 buffer

Multi-MACS stand and MidiMACS Separator	Miltenyi Biotec	130-042-301	Cell isolation
O2k-Fluo Smart-Module	Oroboros	12100-03	
O2k-FluoRespirometer series J	Oroboros	10201-03	
O2k-sV-Module (0.5 chamber)	Oroboros	11200-01	
Oligomycin	Sigma-Aldrich	04876	Fluorespirometry
Pan T Cell Isolation Kit II, mouse	Miltenyi	130095130	Isolation of T-cells from mouse spleen protocol
Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )	Sigma-Aldrich	P0662	Mir05 buffer
Potassium Hydroxide (KOH)	Sigma-Aldrich	221473	Mir05 buffer
Prism	GraphPad	Version 10	
Rotenone	Sigma-Aldrich	R8875	Fluorespirometry
RPMI Buffer	Corning	17-105-CV	Cell isolation
Sodium Pyruvate	Sigma-Aldrich	P2256	Fluorespirometry
Succinate disodium salt	Sigma-Aldrich	S2378	Fluorespirometry
Taurine	Sigma-Aldrich	T0625	Mir05 buffer
Tetramethylrhodamine methyl ester perchlorite	Sigma-Aldrich	T5428	Fluorespirometry