

Materials List for

Real-Time Analysis of Bioenergetics in Primary Human Retinal Pigment Epithelial Cells Using High-Resolution Respirometry

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Materials

Name	Company	Catalog Number	Comments
2-Deoxy-D-glucose	Sigma	D8375-5G	Reconstituted in the Glycolytic Assay Media from its powder form and used in the Glycolytic Stress Test (Port C injection)
96 Well Non-Treated Plate, 5/Pack, Sterile	VWR	229597	BCA Assay 96-well plate
Antimycin A from Streptomyces sp.	Sigma	A8674-25MG	Inhibitor of Complex III of the electron transport chain (part of the Port C drug injection together with rotenone)
BAM15	Sigma	SML1760-5MG	Uncoupling agent used for the Mito Stress Test (Port B injection)
BCA Assay	ThermoFisher	23225	To determine total protein content in each well
Calibrant Solution	Seahorse Bioscience	100840-000	Solution used to calibrate the probes of the sensor cartridge
Cell Lysis buffer 10x	Cell Signaling Technologies	9803S	Dilute 10x Cell Lysis Buffer to a 1x solution using deionized water and add 1 mM PMSF before use
D-(+)-Glucose solution	Sigma	G8644-100ML	Supplement to be added to the Mito Stress Test assay media and also used in the Glycolytic Stress Test (Port A injection)
DMSO, Cell culture grade	Sigma-aldrich	D4540-100ML	For reconstituting all mitochondrial drugs except for 2DG
FCCP	Sigma	C2920-10MG	Uncoupling agent used for the Mito Stress Test (Port B injection)
GlutaMAX	Gibco	35050061	Supplement to be added to the assay media for both the Mito and Glycolytic Stress Tests
HEPES solution	Sigma	H0887-100ML	Supplement to be added to the assay media for both the Mito and Glycolytic Stress Tests

H-RPE – Human Retinal Pigment Epithelial Cells	Lonza	194987	Primary human fetal RPE cells
Oligomycin - CAS 1404-19-9 - Calbiochem	Sigma	495455-10MG	ATP synthase inhibitor used for the Mito Stress Test (Port A injection) and Glycolytic Stress Test (Port B injection)
Parafilm	Bemis	PM996	To seal the plate once the cells are lysed to prevent evaporation in the freezer
Phenylmethylsulfonyl fluoride (PMSF) Protease Inhibitor	Gold Biotechnology Inc	50-153-2823	Used at 1 mM for the 1x Cell Lysis Buffer solution
ReagentPack Subculture Reagents, 100 mL	Lonza	CC-5034	Passaging reagents for primary human fetal RPE cells. Each kit contains 100 mL Trypsin/EDTA, Trypsin Neutralizing Solution, HEPES Buffered Saline
Rotenone	Sigma	R8875-1G	Inhibitor of Complex I of the electron transport chain (part of the Port C drug injection together with antimycin A)
RtEGM Retinal Pigment Epithelial Cell Growth Medium BulletKit - RtEBMTM Basal Medium (00195406) and RtEGMTM SingleQuots Supplements (00195407)	Lonza	195409	Media for primary human fetal RPE cells
Seahorse XFe96 Analyzer	Agilent		High-Resolution Respirometry Instrument
Seahorse XFe96 FluxPak	Agilent	102416-100	This contains the 96-well Seahorse Cell Culture Microplate, the sensor cartridge and the calibrant solution
Sodium pyruvate solution	Sigma	S8636-100ML	Supplement to be added to the Mito Stress Test assay media
Steriflip-GP Sterile Centrifuge Tube Top Filter Unit, 50 mL process volume, 0.22 µm pore size	Millipore-Sigma	SCGP00525	Tube top filter unit for sterile filtration of the assay media
Synergy H1 Plate Reader	BioTek		Plate Reader for measuring absorbance at 562 nm for the BCA assay
XF base medium without phenol red	Agilent	103335-100	Base media for running the Seahorse assay