

PARAMETERS	METHOD	MOLECULAR MARKER	ASSAY SUMMARY	MATERIALS
Cell viability and rate of death	Flow cytometry	Phosphatidylserine (<i>Annexin V</i>) DNA (<i>7AAD</i>)	<i>Annexin V</i> is measured via PE channel (585-42 nm) and <i>7AAD</i> is measured via PC5.5 (690-50 nm)	<i>PE annexin V Apoptosis Detection Kit (BD Pharmingen)</i>
Cell cycle	Flow cytometry	BrDU (<i>anti APC-BrDU</i>) DNA (<i>7AAD</i>)	Anti-BrDU fluorophore is measured via APC channel (660-20 nm) and <i>7AAD</i> is measured via PC5.5 (690-50 nm)	<i>APC BrdU Flow kit (BD Pharmingen)</i>
Mitochondrial levels	Flow cytometry	Mitochondrial mass	Live cells are stained with a dye that accumulates in the mitochondria, then measured via FITC channel (516 nm).	<i>MitoTracker Green FM (Invitrogen)</i>
Autophagy rate levels	Flow cytometry	Monodansyl cadaverine (<i>MDC</i>)	<i>MDC</i> is marked with a fluorophore and then measured via FITC channel (525-40 nm) <i>Rapamycin</i> is used as a positive control.	<i>Autophagy Assay Kit ab139484 (Abcam)</i>
	Western Blot	LC3B	Proteins extracts are obtained with lysis buffer and separated by electrophoresis. Samples are transferred to a PVDF membraned and incubated with antibody. Visualisation is performed using ECL at 16 kDa	3868 <i>(Cell Signaling Technology)</i>
	Quantitative Real Time PCR	<i>MAP1LC3B</i> <i>MFN2</i> <i>PINK1</i> <i>ULK1</i>	cDNA samples were obtained from mRNA by retrotranscription, amplified real time PCR and analysed using the $2^{-\Delta\Delta CT}$ method (RT-qPCR).	<i>Hs00917682_m1</i> <i>Hs00208382_m1</i> <i>Hs00260868_m1</i> <i>Hs00177504_m1</i> <i>(ThermoFisher)</i>
Oxidative mitochondrial state	Flow cytometry	Superoxide radicals in the mitochondria	<i>MitoSOX Red</i> oxidises with mitochondrial superoxide and the fluorescence is measured via ECD channel (610 nm).	<i>MitoSOX (Invitrogen)</i>
	Western Blot	Mn-SOD (<i>SOD2</i>)	Proteins extracts are obtained with lysis buffer and separated by electrophoresis. Samples are transferred to a PVDF membraned and incubated with antibody. Visualisation is performed using ECL at 22 kDa	06-984 (<i>Merck</i>)

	Quantitative Real Time PCR	<i>SOD1</i> <i>SOD2</i> <i>NFE2L2</i> <i>G6PD</i>	RT-qPCR, as before	<i>Hs00167309_m1</i> <i>Hs00167309_m1</i> <i>Hs00232352_m1</i> <i>Hs00166169_m1</i> (ThermoFisher)
Cell hydrogen peroxide levels and antioxidants	Flow cytometry	H ₂ O ₂	Cells are stained prior to treatment and then measured via Pacific Blue channel (450 nm)	<i>Cell Meter Intracellular Fluorimetric Hydrogen Peroxide</i> (AAT Bioquest)
	Quantitative Real Time PCR	<i>CAT</i> <i>GPX1</i> <i>PRDX1</i> <i>PRDX4</i> <i>PRDX5</i> <i>TXN</i> <i>TXN2</i>	RT-qPCR, as before	<i>Hs00156308_m1</i> <i>Hs00829989_gH</i> <i>Hs00602020_mH</i> <i>Hs01056076_m1</i> <i>Hs00201536_m1</i> <i>Hs00828652_m1</i> <i>Hs00429399_g1</i> (ThermoFisher)
Mitochondrial homeostasis and energy production	Quantitative Real Time PCR	<i>ATP5B</i> <i>MYC</i> <i>POLRMT</i> <i>TFAM</i> <i>TFB1M</i>	RT-qPCR, as before	<i>Hs00969569_m1</i> <i>Hs00153408_m1</i> <i>Hs04187596_g1</i> <i>Hs01082775_m1</i> <i>Hs01084404_m1</i> (ThermoFisher)
	SeaHorse	Complex I (<i>Rotenone</i>) Complex III (<i>Antimycin A</i>) Complex V (<i>Oligomycin</i>) Proton gradient in mitochondrial membranes (<i>FCCP</i>)	Cells are treated with modulators of respiration at different time points to measure different aspects of mitochondrial performance	<i>Agilent SeaHorse</i>
	Western Blot	Electron Transport Chain (Complex I to V)	Proteins extracts are obtained with lysis buffer and separated by electrophoresis. Samples are transferred to a PVDF membraned and incubated with antibody. Visualisation is performed via fluorescence from 10 to 50 kDa	<i>OXPHOS</i> <i>45-8199 (Invitrogen)</i>