

Materials List for

# Exploring Mitochondrial Energy Metabolism of Single 3D Microtissue Spheroids Using Extracellular Flux Analysis

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## Materials

| Name  | Company                        | Catalog Number | Comments   |
|---|--------------------------------|----------------|--|
| A549  | ECACC                          | #86012804      | Lung carcinoma cell line   |
| Agilent Seahorse XF RPMI Medium, pH 7.4               | Agilent Technologies Inc.      | 103576-100     | XF assay medium with 1 mM HEPES, without phenol red, sodium bicarbonate, glucose, L-glutamine, and sodium pyruvate |
| Agilent Seahorse XFe96 Extracellular Flux Analyzer    | Agilent Technologies Inc.      | -              | Instrument for measuring rates of spheroid oxygen uptake in single spheroids                                       |
| Antimycin A   | Merck Life Science             | A8674          | Mitochondrial respiratory complex III inhibitor  |
| BAM15   | TOCRIS bio-technie             | 5737           | Mitochondrial protonophore uncoupler   |
| Black-walled microplate                               | Greiner Bio-One                | 655076         | For fluorescence-based assays  |
| CELLSTAR cell-repellent surface 96 U well microplates | Greiner Bio-One                | 650970         | Microplates for generating spheroids   |
| CellTiter-Glo 3D Cell Viability Assay                 | Promega                        | G9681          | Assay for the determination of cell viability in 3D microtissue spheroids  |
| Cultrex Poly-D-Lysine                                 | R&D Systems a biotechnie brand | 3439-100-01    | Molecular cell adhesive for coating XFe96 spheroid microplates to facilitate attachment of spheroids               |
| D-(+)-Glucose   | Merck Life Sciences            | G8270          | Supplement for cell culture growth and XF assay medium   |
| Dulbecco's Modified Eagle Medium (DMEM)               | Gibco                          | 11885084       | Culture medium for HepG2/C3A spheroids   |
| EVOS XL Core Imaging System                           | Thermo Fisher Scientific       | AMEX1000       | Phase-contrast imaging microscope  |
| EZ-PCR Mycoplasma test kit                            | Biological Industries          | 20-700-20      | Mycoplasma screening in cell cultures  |
| FIJI Is Just Image J                                  |                                |                | Analysis of collated images  |
| Foetal bovine serum                                   | Merck Life Science             | F7524          | Supplement for cell culture medium   |
| HepG2/C3A   | ATCC                           | #CRL-10741     | Hepatic carcinoma cell line, a clonal derivative of the parent HepG2 cell line                                     |

|   |                            |                        |  |
|---|----------------------------|------------------------|--|
| Lactate-Glo   | Promega                    | J5021                  | Assay for measurement of lactate within spheroid culture medium  |
| L-glutamine (200 mM solution)                           | Merk Life Sciences         | G7513                  | Supplement for cell culture growth and XF assay medium   |
| M50 Stereo microscope                                   | Leica Microsystems         | LEICAM50               | Stereo dissection microscope; used for spheroid handling   |
| MCF-7   | ECACC                      | #86012803              | Breast adenocarcinoma cell line  |
| Oligomycin from <i>Streptomyces diastatochromogenes</i> | Merck Life Science         | O4876                  | ATP Synthase Inhibitor   |
| Penicillin-Streptomycin                                 | Gibco                      | 15140122               | Antibiotics added to cell culture medium   |
| Quant-iT PicoGreen dsDNA Assay Kit                      | Invitrogen                 | P7589                  | Analysis of dsDNA in spheroids   |
| Rotenone  | Merck Life Science         | R8875                  | Mitochondrial Respiratory Complex I Inhibitor  |
| RPMI 1640   | Gibco                      | 21875091               | Culture medium for A549, MCF7, and SK-OV-3 spheroids   |
| Seahorse Analytics                                      | Agilent Technologies Inc.  | Build 421              | <a href="https://seahorseanalytics.agilent.com">https://seahorseanalytics.agilent.com</a>  |
| Seahorse XFe96 Spheroid FluxPak                         | Agilent Technologies Inc.  | 102905-100             | Each Seahorse XFe96 Spheroid FluxPak contains: 6 Seahorse XFe96 Spheroid Microplates (102978-100), 6 XFe96 sensor cartridges, and 1 bottle of Seahorse XF Calibrant Solution 500 mL (100840-000) |
| Serological pipette: 5, 10, and 25 mL                   | Greiner Bio-One            | 606107; 607107; 760107 | Consumables for cell culture   |
| SK-OV-3   | ECACC                      | #HTB-77                | Ovarian adenocarcinoma cell line   |
| Sodium pyruvate (100 mM solution)                       | Merck Life Science         | S8636                  | Supplement for cell culture growth and XF assay medium   |
| T75 cm <sup>2</sup> cell culture flask                  | Greiner Bio-One            | 658175                 | Tissue culture treated flasks for maintaining cell cultures  |
| TrypLEExpress   | Gibco                      | 12604-021              | Cell dissociation reagent  |
| Wave controller software                                | Agilent Technologies Inc.  | -                      |  |
| Wide orifice tip  | STARLAB International GmbH | E1011-8400             | Pipette tips with wide opening for spheroid handling   |