

## Materials List for:

## Handling and Assessment of Human Primary Prostate Organoid Culture

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## **Materials**

| Name   | Company                 | Catalog Number     | Comments   |
|--|-------------------------|--------------------|--|
| Cells of interest  |                         |                    |  |
| Keratinocyte-SFM (KSFM)  | ThermoFisher Scientific | 17005042           |  |
| Fetal Bovine Serum, charcoal stripped, USDA-approved regions (FBS) | ThermoFisher Scientific | 12676029           |  |
| 5α-Dihydrotestosterone (DHT)                                       | Sigma-Aldrich           | D-073-1ML          |  |
| Flat bottom, polystyrene 96-well cell culture treated plate        | ThermoFisher Scientific | 161093             |  |
| Matrigel (matrix gel)  | Corning                 | Various            | Matrix-gel: growth Factor Reduced,<br>Phenol-red free, etc. depending on<br>application  |
| Ice Bucket   |                         |                    |  |
| Cell Culture Hood  |                         |                    |  |
| 1.5 mL micro-centrifuge tubes or 15 mL conical                     | ThermoFisher Scientific | 05-408-130, 339650 |  |
| Centrifuge   |                         |                    |  |
| Dispase (neutral protease)   | STEMCELL Technologies   | 7923               | neutral protease   |
| Hanks' balanced salt solution (HBSS)                               | ThermoFisher Scientific | 14025076           |  |
| TrypLE Express (cell dissociation enzymes)                         | ThermoFisher Scientific | 12605036           | Cell dissociation enzymes (trypsin may also work, depending on cell type, but TrypLE is more gentle and recommended for primary cells) |
| TRIzol   | ThermoFisher Scientific | 15596018           | suggested RNA extraction solution  |
| RIPA Lysis and Extraction Buffer                                   | ThermoFisher Scientific | 89900              | suggested protein extraction solution  |
| DNAzol   | ThermoFisher Scientific | 10503027           | suggested DNA extraction solution  |
| Organoids  |                         |                    |  |
| Flat bottom, polystyrene 96-well cell culture treated plate        | ThermoFisher Scientific | 161093             |  |
| Brightfield microscope with camera capabilities                    |                         |                    | ex. EVOS FL Auto Imaging System  |
| Photo analysis software  |                         |                    | ex. Photoshop, CELLESTE, ImageJ, MorphoLibJ, CellProfiler  |
| Graphing software  |                         |                    | ex. Graphpad, Excel, etc   |
| Organoids  |                         |                    |  |
| Ice pack   |                         |                    |  |

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|  | <u> </u>                  |                 | 1   |
|--|---------------------------|-----------------|---|
| Masking tape                                     |                           |                 |   |
| Pipette tips (1000 μL)                           |                           |                 |   |
| Razor blade                                      |                           |                 |   |
| Dispase  | STEMCELL Technologies     | 7923            |   |
| Agarose  | Sigma-Aldrich             | A9045           |   |
| HistoGel (histology-gel)                         | ThermoFisher Scientific   | HG-4000-012     |   |
| Pencil   |                           |                 |   |
| "Plunger" from 1 cc insulin syringe              |                           |                 |   |
| Tissue Casette                                   | Thomas Scientific         | 1202D72         |   |
| Container to hold fixative                       |                           |                 |   |
| 10% neutral buffered formalin (NBF)              | Sigma-Aldrich             | HT501128        |   |
| Histology pen, xylene and EtOH-resistant         | Sigma-Aldrich             | Z648191-12EA    | STATMARK pen  |
| Ethanol (histologic grade)                       | Fisher Scientific         | A405P-4         | For fixation and graded dilutions during processing |
| Xylenes  | Sigma-Aldrich             | 214736-1L       |   |
| Deionized water                                  |                           |                 |   |
| Paraffin   | Leica                     | various         |   |
| Tissue processor                                 |                           |                 |   |
| Embedding workstation                            |                           |                 |   |
| Economy Tissue Float Bath                        | Daigger                   | EF4575E XH-1001 |   |
| Microtome  |                           |                 |   |
| Microtome blades                                 | Ted Pella                 | 27243           |   |
| Positively charged microscope slides             | Thomas Scientific         | 1158B91         |   |
| Laboratory Oven                                  | ThermoFisher Scientific   | PR305225G       |   |
| Hematoxylin and Eosin Stain Kit                  | Vector Laboratories       | H-3502          | Suggested H&E staining kit                          |
| ABC Peroxidase Standard Staining Kit             | ThermoFisher Scientific   | 32020           | Suggested immunohistochemistry staining kit         |
| Androgen Receptor Primary<br>Antibody (AR)       | Cell Signaling Technology | 5153S           | Suggested primary antibody for IHC                  |
| FFPE, sectioned organoid sample baked on a slide |                           |                 |   |
| Ethanol (histologic grade)                       | Fisher Scientific         | A405P-4         | dilutions should be performed using deinoized water |
| Xylenes  | Sigma-Aldrich             | 214736-1L       |   |
| Deionized water (DI H <sub>2</sub> O)            |                           |                 |   |
| Antigen retrieval solution                       | Sigma-Aldrich, Abcam      | C9999, ab93684  |   |
| 1x Phosphate Buffered Saline - PBS               | ThermoFisher Scientific   | 10010023        |   |
| Triton X-100                                     | Sigma-Aldrich             | X100-1L         |   |
| Normal Horse Serum                               | thermoFisher Scientific   | 31874           |   |
| Bovin Serum Albumin                              | Sigma-Aldrich             | A2058           |   |
| Counterstain (DAPI, Hoescht)                     | Sigma-Aldrich             | D9542           |   |
| Sodium azide                                     | Sigma-Aldrich             | S2002           | suggested for storage but not required              |
| Confocal microscope                              |                           |                 |   |
| Coplin   | Fisher Scientific         | 19-4            |   |

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|---|--------------------------------|------------|---|
| Staining rack                           | IHC World                      | M905-12DGY |   |
| Staining dish                           | IHC World                      | M900-12B   |   |
| Decloaking chamber                      | Biocare Medical                | DC2012     |   |
| Humidity chamber                        | Thomas Scientific              | 1219D68    |   |
| Hydrophobic barrier pen                 | Vector Laboratories            | H-4000     |   |
| Cytokeratin 8/18 primary antibody (CK8) | ARP American Research Products | 03-GP11    | suggested primary antibody for IF   |
| p63-alpha antibody (p63)                | Cell Signaling Technology      | 4892S      | suggested primary antibody for IF   |
| Keratin 5 Polyclonal Antibody<br>(CK5)  | Biolegend                      | 905501     | suggested primary antibody for IF   |
| Goat anti-rabbit secondary              | ThermoFisher Scientific        | A21245     | suggested secondary antibody for p63 or CK5 detection (do not use at same time) |
| Goat anti-guinea pig secondary          | ThermoFisher Scientific        | A-11075    | suggested secondary antibody for CK8 detection                                  |
| Microscope cover glass                  | Globe Scientific               | 1414-10    |   |
| Anti-fade mounting media                | ThermoFisher Scientific        | S36972     |   |
| Organoids                               |                                |            |   |
| Pipette tips (1000 μL)                  |                                |            |   |
| Pipette tips (200 μL)                   |                                |            |   |
| 8-well chamber slide                    | ThermoFisher Scientific        | 154534PK   | It is also possible to use a confocal dish, depends on preference of user       |
| Cell-Tak                                | Corning                        | CB40240    | optional adherent reagent   |
| 1x Phosphate Buffered Saline (PBS)      | ThermoFisher Scientific        | 10010023   |   |
| 4% paraformaldehye (PFA)                | Biotium                        | 22023      | other fixatives such as methanol or formalin can be used                        |
| 50mM NH <sub>4</sub> CI                 | sigma-Aldrich                  | 254134     |   |
| Triton™ X-100 (non-ionic detergent)     | sigma-Aldrich                  | X100-1L    |   |
| Normal Horse Serum (NHS)                | thermoFisher Scientific        | 31874      |   |
| Bovin Serum Albumin (BSA)               | sigma-Aldrich                  | A2058      |   |
| Counterstain (DAPI, Hoescht)            | Sigma-Aldrich                  | D9542      |   |
| Counterstain (phalloidin)               | thermoFisher Scientific        | A22287     |   |
| Sodium azide                            | sigma-Aldrich                  | S2002      | suggested for storage but not required  |
| Confocal microscope                     |                                |            |   |
| Cytokeratin 8/18 primary antibody (CK8) | ARP American Research Products | 03-GP11    | suggested primary antibody for IF   |
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| Goat anti-guinea pig secondary          | ThermoFisher Scientific        | A-11075    | suggested secondary antibody for CK8 detection                                  |
| Visikol HISTO-M                         | Visikol                        | various    | optional clearing agent   |
| Organoids                               |                                |            |   |
| Pipette tips (1000 μL)                  |                                |            |   |

| Pipette tips (200 μL)                      |                         |           |  |
|--|-------------------------|-----------|--|
| 8-well chamber slide                       | ThermoFisher Scientific | 154534PK  | It is also possible to use a confocal dish, depends on preference of user  |
| Cell-Tak                                   | Corning                 | CB40240   |  |
| 1x Phosphate Buffered Saline - PBS         | ThermoFisher Scientific | 10010023  |  |
| Cell assay of interest                     | Various                 | Various   | Click-iT EdU Alexa Fluor proliferation assay (fluorescently-labelled EdU proliferation kit), Image-iT Lipid Peroxidation Kit, etc) and fluorescent probes/dyes (ex. HCS mitochondrial Health Kit, CellMask, LIVE/DEAD Viability assays, CellROX reagents, etc) |
| Organoids                                  |                         |           |  |
| Ice bucket                                 |                         |           |  |
| Cell culture hood                          |                         |           |  |
| 1.5 mL eppendorf tubes or 15 mL conical    |                         |           |  |
| Microcentrifuge                            |                         |           |  |
| Dispase                                    | STEMCELL Technologies   | 7923      |  |
| TrypLE Express (cell dissociation enzymes) | ThermoFisher Scientific | 12605036  | Cell dissociation enzymes (trypsin may also work, depending on cell type, but TrypLE is more gentle and recommended for primary cells)   |
| Hanks' balanced salt solution (HBSS)       | ThermoFisher Scientific | 14175079  |  |
| Flow Tube with Cell Strainer Snap<br>Cap   | Fisher Scientific       | 08-771-23 |  |
| Cytokeratin 5 Antibody - FITC              | Millipore Sigma         | FCMAB291F | Suggested flow antibody  |
| Cytokeratin 8 Antibody - Alexafluor<br>405 | Abcam                   | ab210139  | Suggested flow antibody  |
| CD49f - Alexafluor 647                     | BioLegend               | 313609    | Suggested flow antibody  |
| CD26 - PE                                  | BioLegend               | 320576    | Suggested flow antibody  |
| Flow cytometer                             |                         |           |  |