

Materials List for:

The Aortic Ring Co-culture Assay: A Convenient Tool to Assess the Angiogenic Potential of Mesenchymal Stromal Cells *In Vitro*

Farwah Iqbal^{1,2}, Yarden S. Gratch¹, Peter Szaraz^{1,2}, Clifford L. Librach^{1,2,3,4,5}

¹Create Fertility Centre

²Department of Physiology, University of Toronto

³Department of Obstetrics and Gynecology, University of Toronto

⁴Department of Medical Sciences, University of Toronto

⁵Department of Obstetrics and Gynecology, Women's College Hospital

Correspondence to: Farwah Iqbal at farwah@createivf.com

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Materials

| Name | Company | Catalog Number | Comments |
|---|-----------------------|----------------|--|
| Alpha-MEM | Gibco | 12571071 | For FTM HUCPVC and BMSC culture media. |
| APC-conjugated anti human TRA-1-85 | R&D Systems | FAB3195A | Human-specific cell marker for flow cytometry and cell sorting |
| Basal membrane extract (BME) (Matrigel) | Corning | 354234 | For aorta embedding |
| Bullet-kit | Lonza | CC-3162 | Includes: Gentamicin/ Amphotericin-B (GA)human Epidermal Growth Factor (hEGF); Vascular Endothelial Growth Factor (VEGF); R3- Insulin-like Growth Factor-1 (R3-IGF-1); Ascorbic Acid; Hydrocortisone; human Fibroblast Growth Factor-Beta (hFGF-β); Heparin; Fetal Bovine Serum (FBS). Required to prepare EGM |
| CellTracker Green CMFDA Dye | Thermo-fisher | C2925 | For staining MSCs, green is picked up optimally by MSCs |
| CKX53 Culture Microscope | Olympus | | For bright-field imaging of endothelial network development |
| Countess automated cell counter | Invitrogen | C10227 | Cell counting for MSC culture, flow cytometry and qPCR |
| Dispase | StemCell technologies | 7923 | For dissociating aortic ring-MSc co-cultures (pre-warm at 37 °C) |
| Disposable sterile scalpels | VWR | 21909-654 | For sectioning aorta |
| Dulbecco's phosphate buffered saline | Sigma-Aldrich | D8537 | PBS. 1X, Without calcium chloride and magnesium chloride |
| Endothelial basal media (EBM) | Lonza | CC-3156 | Basal media required for culturing aortic ring assay-MSc co-cultures (warm at 37 °C before use). Required for EGM and EBM-FBS |
| Ethanol, 70%, Biotechnology Grade | VWR | 97064-768 | To sterilize surfaces |
| EVOS | Life Technologies | | In-house fluorescent microscope to track MSc migration and integration |

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|---|--------------------------|--------------|---|
| Fetal bovine serum (FBS) (Hyclone) | GE Healthcare | SH3039603 | Serum component of cell culture medium |
| FITC-conjugated anti-CD31 antibody | BD | 558068 | Human endothelial marker for flow cytometry |
| FITC-conjugated anti-CD146 antibody | BD | 560846 | Human pericyte marker for flow cytometry |
| Forceps | Almedic | 7727-A10-704 | For handling rat tissue. Can use any similar forceps |
| Hank's Balanced Salt Solution (HBSS) | Life Technologies | 14175-094 | 1X Without calcium chloride and magnesium chloride |
| HERAcell 150i CO ₂ Incubator | Thermo Fisher Scientific | 51026410 | For incubating cells |
| Human Angiogenesis RT2 profiler PCR array | Qiagen | PAHS-024Z | Human specific and includes primers for 84 genes involved in angiogenesis. Each well is 1 primer reaction |
| ImageJ | | | Open source image processing software. Require Angiogenesis analyzer plugin |
| LSR II | BD | | UHN SickKids FC Facility. For flow cytometry. |
| MoFlo Astrios | Beckman Coulter | | UHN SickKids FC Facility. For cell sorting. |
| Penicillin/streptomycin | Gibco | 15140122 | Antibiotic component to buffers and cell culture medium |
| RNeasy Mini Kit | Qiagen | 74104 | For RNA purification. Includes cell lysis buffer |
| RT ² Easy First Strand Kit | Qiagen | 330421 | For preparation of cDNA for qPCR |
| RT ² PreAMP cDNA Synthesis Kit | Qiagen | 330451 | Pre-amplification of cDNA if low-yield RNA |
| Surgical scissors | Fine Science Tools | 14059-11 | For cutting skin, muscle and aorta |
| Sterile gauze | VWR | 3084 | To dampen and sterilize chest fur |
| TrypleE | Thermo Fisher Scientific | 12605036 | MSC dissociation enzyme pre-warm at 37 °C |
| 0.2 µm pore filtration unit | Thermo Fisher Scientific | 566-0020 | To sterilize tissue culture media |
| 0.25% Trypsin/EDTA | Gibco | 25200056 | For cell dissociation, pre-warm at 37 °C |
| 10 cm tissue culture dishes | Corning | 25382-428 | For cleaning and sectioning aorta and MSC cell culture |
| 12 well-cell culture plates | Corning-Sigma Aldrich | CLS3513 | For setting up aortic ring assay-MSC co-cultures |
| 15 mL tube | BD Falcon | 352096 | For general tissue culture procedures |
| 70 µm cell strainer | Fisherbrand | 22363548 | To ensure a single cell suspension before flow cytometry or sorting |