Materials List for
Isolation of Adipogenic and Fibro-Inflammatory Stromal Cell Subpopulations from Murine Intra-Abdominal Adipose Depots

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Materials

<table>
<thead>
<tr>
<th>Name</th>
<th>Company</th>
<th>Catalog Number</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical Tissue Preparation and SVF Isolation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 and 100 µm cell strainers</td>
<td>Fisher Scientific</td>
<td>352340/352360</td>
<td></td>
</tr>
<tr>
<td>1X Phosphate buffered saline (PBS)</td>
<td>Fisher Scientific</td>
<td>21040CV</td>
<td></td>
</tr>
<tr>
<td>5ml polypropylene tubes</td>
<td>Fisher Scientific</td>
<td>352053</td>
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</tr>
<tr>
<td>Digestion Buffer (for 10mL)</td>
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</tr>
<tr>
<td>10 ml HBSS</td>
<td>Sigma</td>
<td>H8264</td>
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</tr>
<tr>
<td>10 mg Collagenase D (1 mg/ml final cc.)</td>
<td>Roche</td>
<td>11088882001</td>
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<tr>
<td>0.15 g BSA (1.5 % final cc.)</td>
<td>Fisher Scientific</td>
<td>BP1605-100</td>
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<tr>
<td>Immunomagnetic separation of APCs and non-APCs</td>
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<tr>
<td>5X MojoSort Buffer (MS buffer)</td>
<td>BioLegend</td>
<td>480017</td>
<td></td>
</tr>
<tr>
<td>5 ml MojoSort Magnet (MS magnet)</td>
<td>BioLegend</td>
<td>480019</td>
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<tr>
<td>100 µL MojoSort Streptavidin Nanobeads</td>
<td>BioLegend</td>
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<td>Purity Check and FACS</td>
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<tr>
<td>10X Red Blood Cell Lysis Buffer</td>
<td>eBioscience</td>
<td>00-4300-54</td>
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<tr>
<td>Fc block (Mouse CD16/CD32)</td>
<td>eBioscience</td>
<td>553141</td>
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<tr>
<td>Antibodies</td>
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<tr>
<td>Biotin CD45</td>
<td>BioLegend</td>
<td>103103</td>
<td>Concentration: ≤ 0.25 µg per 10^6 cells Species: Mouse Clone: 30-F11</td>
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<tr>
<td>Biotin CD31</td>
<td>BioLegend</td>
<td>102503</td>
<td>Concentration: ≤ 0.25 µg per 10^6 cells</td>
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<tr>
<td>Antibody</td>
<td>Supplier</td>
<td>Catalog Number</td>
<td>Concentration/Species(Clone)</td>
</tr>
<tr>
<td>----------</td>
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</tbody>
</table>
| Biotin CD9 | BioLegend | 124803 | Concentration: ≤ 0.25 µg per 10^6 cells  
Species: Mouse  
Clone: MEC13.3 |
| Biotin LY6C | BioLegend | 128003 | Concentration: ≤ 0.25 µg per 10^6 cells  
Species: Mouse  
Clone: MZ3 |
| CD31-PerCP/Cy5.5 | BioLegend | 102419 | Concentration: Dilution 1:400  
Species: Mouse  
Clone: HK1.4 |
| CD45-PerCP/Cy5.5 | BioLegend | 103131 | Concentration: Dilution 1:400  
Species: Mouse  
Clone: 30-F11 |
| CD140b PDGFRβ-PE | BioLegend | 136006 | Concentration: Dilution 1:50  
Species: Mouse  
Clone: APB5 |
| LY6C-APC | BioLegend | 128016 | Concentration: Dilution 1:400  
Species: Mouse  
Clone: MZ3 |
| CD9-FITC | BioLegend | 124808 | Concentration: Dilution 1:400  
Species: Mouse  
Clone: MZ3 |

**Cell Culture and Differentiation**

**Gonadal APC Culture media (for 500mL)**

- **288 mL DMEM with 1 g/L glucose**  
  Supplier: Coming  
  Catalog Number: 10-014-CV
- **192 mL MCDB201**  
  Supplier: Sigma  
  Catalog Number: M6770
- **10 mL Fetal bovine serum (FBS)**  
  Supplier: Sigma  
  Catalog Number: 12303C
- **5 mL 100% ITS premix**  
  Supplier: BD Bioscience  
  Catalog Number: 354352
- **5 mL 10 mM L-ascorbic acid-2-phosphate**  
  Supplier: Sigma  
  Catalog Number: A8960-5G
- **50 µL 100 g/ml FGF-basic**  
  Supplier: R&D systems  
  Catalog Number: 3139-FB-025/CF
- **5 mL Pen/Strep**  
  Supplier: Coming  
  Catalog Number: 30-001-CI
- **500 µL Gentamycin**  
  Supplier: Gibco  
  Catalog Number: 15750-060

**NOTE:** The adipogenic capacity of primary APCs can vary from lot to lot of commercial FBS. Multiple lots/sources of FBS should be tested.