1.0 Purpose
The purpose of this modified SOP 2.4 is to describe how to dissociate cells from mouse xenograft tumor.

2.0 Scope
SOP 2.4 is intended to cover all resources, personnel and equipment in the BCR laboratory.

3.0 Materials

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Description</th>
<th>Storage Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>CO₂</td>
<td>Carbon Dioxide</td>
<td>026-374C</td>
</tr>
<tr>
<td>2.0</td>
<td>EtOH</td>
<td>Ethanol</td>
<td>Fridge by hood</td>
</tr>
<tr>
<td>3.0</td>
<td>Collagenase</td>
<td>Enzymatic Dissociation</td>
<td>Freezer #2 (026-328S-A)</td>
</tr>
<tr>
<td>4.0</td>
<td>HBSS</td>
<td>Hank’s Balanced Salt Solution</td>
<td>Cold Storage (026-380C)</td>
</tr>
<tr>
<td>5.0</td>
<td>RPMI 1640/10%FBS</td>
<td>Complete Medium</td>
<td>Cold Storage (026-380C)</td>
</tr>
<tr>
<td>6.0</td>
<td>Trypsin</td>
<td>Protease</td>
<td>Freezer #2 (026-328S-A)</td>
</tr>
<tr>
<td>7.0</td>
<td>Cell Strainer (BD Falcon)</td>
<td>100uM Nylon</td>
<td>Supply Area (026-328S-A)</td>
</tr>
<tr>
<td>8.0</td>
<td>Cell Strainer (BD Falcon)</td>
<td>40uM Nylon</td>
<td>Supply Area (026-328S-A)</td>
</tr>
<tr>
<td>9.0</td>
<td>Collagenase/Hyaluronidase</td>
<td>SCT cat# 07912, Enzyme</td>
<td>Freezer #2 (026-328S-A)</td>
</tr>
</tbody>
</table>

4.0 Procedure

Collagenase Digestion

4.1 Euthanize mouse with isoflurane and CO₂ and place it into 70% EtOH in a white bucket, and transfer to hood.
- Move mice to paper towel prior to dissection.

4.2 Take out fresh xenograft tumor tissue from mouse and place into labeled Petri dish (10 cm plate) for dissociation. *If the sample is too large for one plate, use multiple Petri dishes and combine after mincing.*

4.3 Cut one piece from the edge and place into corresponding cassette for Histology. Place cassette in 10% formalin.
- If no tumor exists, remove fatpad and place in cassette for Histology.

4.4 Mince remaining tissue into very fine pieces by crossly using two sterile scalpels. Transfer the minced tissue into a 50-ml Conical tube, and add 10-15ml warm tumor Collagenase solution.

4.5 Either shake the tube on a rotary shaker for 2-3 hours and check per 30 minutes until all larger tissue fragments are digested OR mix with a 5 mL pipette and place 50 mL tube(s) into a 37 degree C water bath, and every 15 minutes pipette each sample with a 5 mL pipette, then place back in water bath for a total of 45 minutes.

4.6 Add HBSS with 2% FBS to the 50 mL tube(s) and sieve each sample through a 40 uM cell filter into second labeled corresponding 50 mL tube.
- Keep washed samples on ICE.

4.7 Spin down at 1000 rpm for 5 minutes. Discard the supernatant.

4.8 Wash in 10 mL HBSS, centrifuge again and then aspirate the supernatant.

4.9 Resuspend pellet in 1 mL or so (depending on pellet size) of HBSS with 2% FBS to count cells.
Trypsin Digestion

4.10 Spin down at 1000 rpm for 5 minutes. Discard the supernatant.
4.11 Wash the pellet with HBSS and Spin down.
4.12 Re-suspend the pellet 3-5ml 0.25% Trypsin and Incubate in 37°C for 5 minutes.
4.13 Add 5ml RPMI 1640/10%FBS to inactivate Trypsin. Add 10ml HBSS.
4.14 Filter with a 100uM Nylon Cell Strainer. Then filter with another 40uM Nylon Cell Strainer.
4.15 Collect the dissociated cells by Centrifuging at 1000 rpm for 5 minutes. Continue to next step or Save in -80°C with the Freezing Medium for future experiments.

Histology Samples in Cassettes:
After 24 hours, transfer cassettes from 10% Formalin into 70% Ethanol (in a biohazard ziplock bag). Make sure all cassettes are covered in ethanol. Fill out appropriate paperwork and take samples to Pathology lab on the 3rd floor CC for sectioning.

**NOTE** Pathology lab closes at 4pm.

5.0 Applicable References