1.0 Purpose
The purpose of SOP 4.30 is to provide information on proper procedure for preparing the three enzymes used to dissociate tumors using Miltenyi gentleMACS dissociator.

2.0 Scope
SOP 4.30 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>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>RPMI 1640</td>
<td>Gibco cat# 11875-093</td>
<td>Cold storage (026-380C)</td>
</tr>
<tr>
<td>2.0</td>
<td>Human tumor digestion kit</td>
<td>Miltenyi cat# 130-095-929</td>
<td>Fridge 1 (026-314S)</td>
</tr>
</tbody>
</table>

4.0 Procedure
4.1 Prepare enzyme H by reconstituting lyophilized powder in each of the 2 vials provided in kit with 3 mL of RPMI each. Aliquot into 1.5 mL Eppendorf tubes at 200 uL each tube.
4.2 Prepare enzyme R by reconstituting lyophilized powder in the vial labeled “R” with 2.7 mL RPMI. Aliquot into .5 mL Eppendorf tubes at 100 uL each.
4.3 Prepare enzyme A by reconstituting lyophilized powder in the vial with 1 mL of Buffer A provided in the kit. Aliquot into .5 mL Eppendorf tubes at 25 uL each.
4.4 Store any unused stock in the freezer for up to 6 months.

5.0 Applicable References

6.0 Change Description