1.0 Purpose
The purpose of SOP 8.2 is to provide details on how to prepare aldefluor in MCF-10 cells for flow cytometry.

2.0 Scope
SOP 8.2 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>HBSS</td>
<td>Hanks Balanced Salt Solution</td>
<td>Cold Storage (026-380C)</td>
</tr>
<tr>
<td>2.0</td>
<td>FBS</td>
<td>Fetal Bovine Serum</td>
<td>Freezer #2 (026-328S-A)</td>
</tr>
<tr>
<td>3.0</td>
<td>BAAA</td>
<td>Activated Aldefluor substrate</td>
<td>Freezer #3 (026-314S-A)</td>
</tr>
<tr>
<td>4.0</td>
<td>DEAB</td>
<td>Inhibitor</td>
<td>Fridge #5 (026-320S-A)</td>
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</tbody>
</table>

4.0 Procedure

4.1 Cells: MCF-10A-Ras, MCF-10A-RasDKO, MCF-10A-RasKO, MCF-10A-RasD53, MCF-10A-RasDBS+D53, MCF-10A-Ras-D53, MCF-10A-RasDBS

4.2 Labeled tubes: DAPI only, BAAA + Inhibitor (Control), BAAA - Inhibitor (Test) for each cell type. Label one "test" and one "control" 12 x 75 mm tube for each sample to be tested.

4.3 Beginning with the first sample, add 5 µL of DEAB (inhibitor) solution to the "control" tube. Recap control tube and DEAB vial immediately.

- DEAB is provided in 95% ethanol. Recap immediately to prevent evaporation!

4.4 Add 5 µL of activated ALDEFLUOR substrate (BAAA) per 10^6 cells (1 mL) to the first sample “test” tube.

4.5 Mix and immediately transfer 0.5 mL of the mixture to the DEAB “control” tube and > 0.1 mL of the mixture to the BAAA only tube.

- The ALDH enzymatic reaction begins immediately upon addition of the activated substrate to the cell suspension. It is imperative that an aliquot of the ALDEFLUOR-reacted cells be added to the DEAB control tube without delay.

4.6 Repeat step 4.3 for each sample to be tested.

4.7 Incubate “test” and “control” samples for 30-60 minutes (40 minutes) at 37° C (Do not exceed 60 minutes)

- If immunophenotyping is performed in addition to the ALDH assay, add and incubate the antibodies after step 4. See the section on Immunophenotyping for recommendations.

4.8 Following incubation, add HBSS with 2% FBS and wash 3 times.

4.9 Then add HBSS with PI to DAPI only, BAAA + Inhibitor (Control) and BAAA – Inhibitor (test) tubes.

4.10 If samples are not analyzed immediately, cap samples and place on ice or in the refrigerator. Samples are stable for 24 hours at 2-8°C.

4.11 Set up the selected flow cytometer instrument per manufacturer’s instructions. Perform data acquisition of each sample. Acquire at least 100,000 events per sample.

5.0 Applicable References

6.0 Change Description

<table>
<thead>
<tr>
<th>Revision</th>
<th>Date</th>
<th>Reference</th>
<th>Description of Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>7/3/12</td>
<td>CL</td>
<td>Added room locations</td>
</tr>
</tbody>
</table>