ALDEFLUOR ASSAY

MATERIALS PROVIDED

ALDEFLUOR Kit (Stemcell Technologies, Cat#: 01700) contains:

Dry ALDEFLUOR reagent, 50 µg

Diethylaminobenzaldehyde (DEAB), 1.5 mM in 95% ethanol, minimum 200 µL

Hydrochloric Acid (HCl), 2N, minimum 50 µL

Dimethylsulphoxide (DMSO), minimum 50 µL

ALDEFLUOR Assay Buffer, 4 bottles of 25 mL each

ALDEFLUOR Quick Reference Guide

Antibodies

CD3-APC, Caltag Laboratories, Cat#: MHCD0305

CD33-PECY5, Beckman Coulter, Cat#: IM2647U

INSTRUCTIONS FOR USE

Reagent Preparation (performed only with initial use of kit)

1. Assemble all necessary supplies and allow kit reagents to come to room temperature (RT), 18 to 22°C before use.
2. Activate the ALDEFLUOR reagent:
   a. Add 25 µL of DMSO to the vial of dry ALDEFLUOR reagent and mix well.
   b. Let stand for 1 min at RT.
   c. Add 25 µL of 2N HCl and mix well.
   d. Incubate this mixture for 15 min at RT. Do NOT exceed 30 minutes.
3. Add 360 µL of ALDEFLUOR Assay Buffer to the vial and mix.

Note: Upon addition of the Assay Buffer, the solution may appear slightly cloudy. This does not affect the assay performance.

4. Keep the activated reagent at 2 to 8°C during use.
5. Any remaining activated ALDEFLUOR substrate should be dispensed into aliquots(50µL-100µL each) and stored frozen at or below -80°C.

Sample Preparation

1. Ficoll zzzz

lyse the erythrocytes after the Ficoll with RPCI standard ammonium chloride-based buffered solution.

2. Revert 3 times. Let stand for 5 min at room temperature.
3. Centrifuge the sample for 5 min at 250 x g, remove the supernatant and suspend cells in 1 mL of ALDEFLUOR Assay Buffer.

   Note: If residual RBC present, a second lysis procedure should be performed. Otherwise the assay will not work.

4. Perform a cell count on ACT10.
5. Adjust sample to a concentration of $1x10^6$ cells/mL with Assay Buffer.

ALDEFLUOR Assay

1. Label one 12 x 75 mm tube "test" and one 12 x 75 mm tube "control" for each sample to be tested. Place 1.0 mL of the adjusted cell suspension into each "test" sample tube.
2. Beginning with the first sample:
   a. Add 5 µL of DEAB solution to the "control" tube. Recap control tube and DEAB vial immediately to prevent evaporation.
   b. Add 5 µL of activated ALDEFLUOR substrate per mL of sample to the first sample "test" tube.

c. Mix and immediately transfer 0.5 mL of the mixture to the correspondingly labeled DEAB "control" tube.

Note: 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.

3. Repeat step 2 for each sample to be tested.
4. Incubate "test" and "control" samples for exactly 45 minutes at 37°C.
5. Following incubation, centrifuge all tubes for 5 min at 250 x g and remove supernatant. Suspend cell pellets in 0.1 mL of ALDEFLUOR Assay Buffer.
6. Add mAbs into each tube. Incubation on ice 30 minutes in dark.

   ALDEFLUOR in FITC channel
   CD3 APC
7. Add 400µL of ALDEFLUOR Assay Buffer to each tube (Final volume will be 500 µL).
8. Centrifuge all tubes for 5 min at 250 x g and remove supernatant. Suspend cell pellets in 0.5 mL of ALDEFLUOR Assay Buffer.
9. If samples are not analyzed immediately, cap samples and place on ice or in the refrigerator. Samples are stable for 24 hours at 2 to 8°C.
10. 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.