ALDAGEN

TECHNOTES

IMMUNOPHENOTYPING OF ALDEFLUOR®-REACTED CELLS

ALDEFLUOR reagent is a non-toxic fluorescent substrate for aldehyde dehydrogenase (ALDH) which has been optimized for the identification of human stem and progenitor cells expressing high levels of ALDH activity (ALDH-bright) by flow cytometry. Immunophenotyping can be performed in conjunction with the ALDEFLUOR assay.

ALDEFLUOR reagent shows an emission spectrum similar to FITC with peak emission at 512 nm. Due to the spectral overlap of the ALDEFLUOR reagent with fluorochromes that are detected below 650 nm, we recommend using antibodies conjugated to fluorochromes that emit at higher wavelengths for antigens which typically exhibit low levels of expression. For example, to study the coexpression of CD34 on ALDH-bright cells we use the antibody combination CD45 phycoerythrin (PE), 7-aminoactinomycin D (7-AAD) and CD34 allophycocyanin (APC). Due to the brightness of the ALDEFLUOR reagent fluorophore, adequate compensation will not be achieved with commercially available fluorescent beads. Cellular compensation controls should be used instead.

Antibody staining is performed subsequent to the ALDEFLUOR assay. In brief, the erythrocytes are lysed (see TSB02), the cell suspension is adjusted to the appropriate concentration and the ALDEFLUOR reaction is performed per the product insert. Antibody conjugates are then added directly to the ALDEFLUOR-reacted cell suspension and the mixture is incubated at 2-8°C. The samples are washed, suspended in assay buffer and analyzed. The details for setting up a 4-color assay, including staining of the proper compensation controls, are provided below.

Materials Required

1. ALDEFLUOR kit
2. Antibodies conjugated to non-FL1 fluorochromes
3. Isotype control for each antibody

Procedure

If using a blood product, the erythrocytes must be lysed before running the ALDEFLUOR reaction.

1. For each specimen, label three 12 x 75 mm tubes with the specimen ID; label one tube for the ALDEFLUOR reaction, one for the control, and one for phenotyping. Label one additional ALDEFLUOR reaction tube for one specimen within each run that will be used for compensation controls. Label four 12 x 75 mm tubes (1 – 4) for compensation controls.
2. Perform the ALDEFLUOR assay per the product insert through step 4.
3. Following the 37°C incubation, place tubes on ice.

4. Add antibodies to 12 x 75 mm tubes. Use Table 1 to determine antibody combinations for the isotype controls.

   Table 1: Compensation matrix.

   For each compensation control tube add the antibodies as indicated by an X. Refer to manufacturer's instructions regarding appropriate amount of antibody to use.

Tube #	1	2	3	4
	FL1 comp	**FL2 comp**	**FL3 comp**	**FL4 comp**
FL2 Isotype control antibody	X		X	X
FL3 Isotype control antibody	X	X		X
FL4 Isotype control antibody	X	X	X	
FL2 Antibody		X		
FL3 Antibody			X	
FL4 Antibody				X
ALDEFLUOR reacted cells (µl)	500	500	500	500

5. For each test sample:

   a) Mix cells and transfer 0.5 ml from each ALDEFLUOR Reagent tube into the appropriate phenotyping tube.
   b) Incubate tubes for 15-30 minutes at 2-8°C.
   c) Add 0.5 ml of ALDEFLUOR Assay Buffer to all tubes.
   d) Centrifuge all test and control tubes at 250 x *g* for 5 minutes.
   e) Remove the supernatant.
   f) Suspend each cell pellet in 0.5 ml of ALDEFLUOR Assay Buffer.

6. Place samples on ice or in the refrigerator. Samples are stable for 24 hours at 2° to 8°C.

Note: ALDEFLUOR Assay Buffer has been optimized for use with human cells. When working with cells from other species, it is critical to maintain cells at 2-8°C during all steps subsequent to the ALDEFLUOR reaction.