ALDAGEN TECHNOTES

PREPARATION OF HUMAN PERIPHERAL BLOOD STEM CELL COLLECTIONS AND WHOLE BLOOD SAMPLES FOR ALDEFLUOR®: ERYTHROCYTE LYSIS USING AMMONIUM CHLORIDE SOLUTION

The large number of erythrocytes present in peripheral blood stem cell collections and whole blood samples competes with stem cells for aldehyde dehydrogenase substrate and therefore can interfere with the detection of stem cells using the ALDEFLUOR® reagent. Such interference can be eliminated by lysing erythrocytes with an appropriate ammonium chloride-based buffered solution before initiating the ALDH assay. Because the ALDH reaction identifies viable functional cells, lysis buffers containing detergents or fixatives are not compatible with the assay. (You may contact Aldagen, Inc to obtain a list of recommended erythrocyte lysis buffers.)

The ammonium chloride procedure given below is a reliable pretreatment for use with peripheral blood cell collections obtained using conventional apheresis procedures or with whole blood samples. This method is time sensitive and the procedure must be followed to obtain acceptable sample lysis. Note that for the ALDH (ALDEFLUOR®) assay, red blood cells must be lysed before staining.

For whole blood and apheresis products, the acceptable erythrocyte to leukocyte ratio is 2:1 or less. This lysis procedure may have to be performed twice on peripheral blood samples to achieve sufficient red cell removal.
Note: Conditions for lysis of erythrocytes from bone marrow and umbilical cord blood samples are different. Protocols for these sample types are available in the ALDECOUNT® Lysis Buffer product insert.

1. With human apheresis or peripheral blood samples, use a volume ratio of 10 parts lysis buffer to 1 part cell suspension. [Example, 10 ml of buffer to 1 ml of cells]
2. Cap the tube(s) and gently mix each cell suspension by inversion immediately after adding the lysing solution.
3. Incubate each cell mixture at room temperature for 15 minutes.
4. Centrifuge @ 250 X g for 5 minutes.
5. Remove the supernatant fluid.
6. Suspend the cells in ALDEFLUOR® assay buffer, adjusting the concentration to approximately 1 x 10^6 leukocytes per ml and proceed with the ALDH staining procedure as outlined in the product package insert.

Please refer to the ALDECOUNT® Lysis Buffer product insert for the proper conditions for lysing erythrocytes in bone marrow and umbilical cord blood.

U.S. Patent No. 5,876,956; 6,627,759; 6,537,807; 6,991,897. Australian Patent No. 774566; 753975. Singapore Patent No. P-81176.

Aldagen, Inc
TSB02(02)