Protocol Optimization for Detecting ALDH-expressing Normal Stem and Progenitor Cells, and Cancer Cells Using ALDEFLUOR®

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Abstract

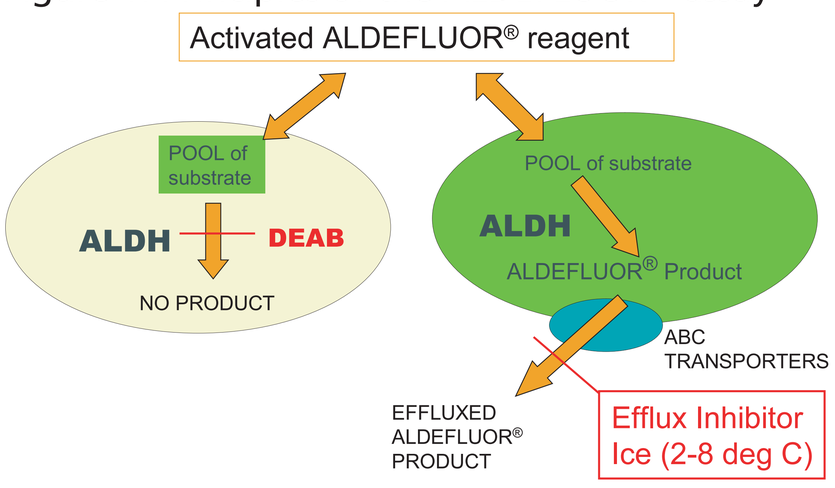
Stem and progenitor cells isolated from various normal tissues, as well as some primary tumors and cancer cell lines have been reported to contain high levels of aldehyde dehydrogenase (ALDH) enzyme activity. ALDH-expressing cells can be quantitated using a commercially-available reagent, ALDEFLUOR® (Aldagen Inc). Cells are treated with Bodipy®-aminoacetaldehyde that freely diffuses into cells and is selectively converted by ALDH to a non-toxic fluorescent compound Bodipy®-aminoacetate that is retained in the cells in the presence of an appropriate ABC transporter inhibitor. Cells treated with DEAB, an ALDH inhibitor, are used as the negative control. ALDH-bright cells are detected by flow cytometry. Cell fluorescence staining profiles may vary with the cell type, fresh vs. cultured cells, cell viability, and assay conditions used. The SKBR3 mammary carcinoma cell line was used to evaluate optimal conditions for detection of ALDH activity. The reaction reagents were titrated to ensure mean fluorescence intensity (MFI) of ALDH-bright cells is ≥2.5-fold higher than DEAB-treated controls. Optimal incubation times were 30-45 minutes at 37°C, with a 20% decrease shown at 60 minutes. Cell concentrations at 1-2 x10^5/mL yielded 3-fold higher MFI compared to the negative control whereas higher concentrations (0.5-2 x10^6/mL) resulted in ~50% lower MFI. Viable intact cells are required for detection of cellular ALDH activity and the use of a viability marker (e.g. propidium iodide) is recommended to exclude non-viable, late apoptotic cells and debris. Using these conditions, ~20-50% ALDH-bright cells were detected in 7-day cultures of CD34+ cord blood (CB) cells in cytokine-supplemented StemSpan® SFEM. The number of ALDH-bright cells was found to be significantly higher if the CB cells were co-cultured on human bone marrow-derived mesenchymal cells using the same culture conditions. This study emphasizes the importance of protocol optimization to obtain accurate, reproducible data on ALDH enzyme activity in different cell populations.

What is ALDEFLUOR®?

ALDEFLUOR® identifies cells on the basis of aldehyde dehydrogenase (ALDH) activity

- Measures an intracellular enzymatic reaction
- Requires viable, living cells
- Non-toxic
- Measured by flow cytometry

Figure 1: Principles of the ALDEFLUOR® assay



Important parameters for detection of ALDH activity in non-hematopoietic or cultured cells

- Cell concentration: 1x10^5-1x10^6 / mL
- ALDEFLUOR® substrate (BAAA) concentration
- ALDEFLUOR® inhibitor (DEAB) concentration
- Different efflux inhibitors; e.g., verapamil, probenecid, sodium azide
- Incubation times: 30-60 minutes are typical
- Cells should be kept **on ice** after incubation
- Time between incubation and analysis should be kept as short as possible
- Quality/viability of cell preparations: low numbers of dead or apoptotic cells; Use a vital dye (e.g., propidium iodide or 7-AAD) to stain and gate out dead and late apoptotic cells

Results

Figure 2: Typical FACS plot of ALDH^{bright} SSC^{low} cells from a hematopoietic source (human mobilized peripheral blood)

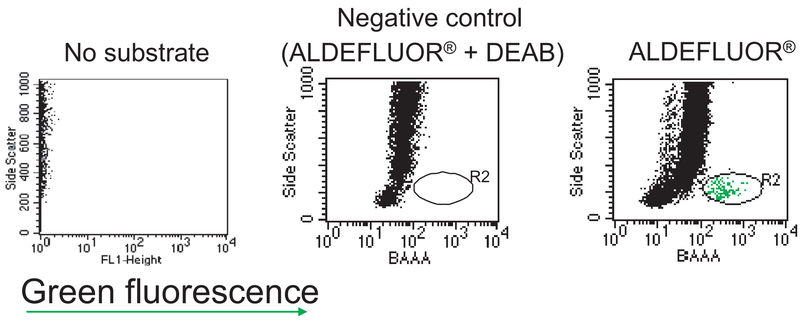
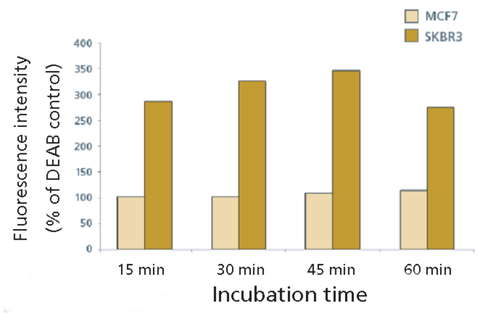
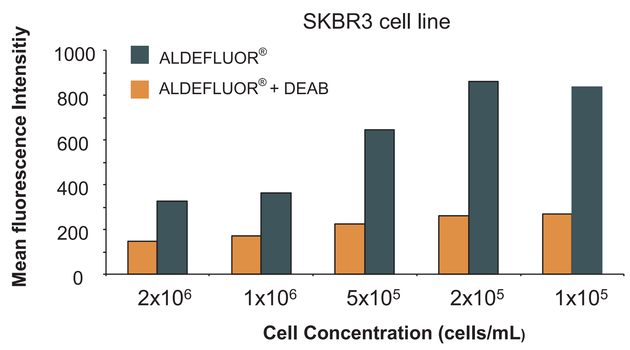


Figure 3: Incubation time



Study using two breast cancer cell lines, SKBR3 (ALDH^{br}) and MCF7 (ALDH^{lo}) shows incubation times of 30-45 min. are optimal.

Figure 4: Cell concentration

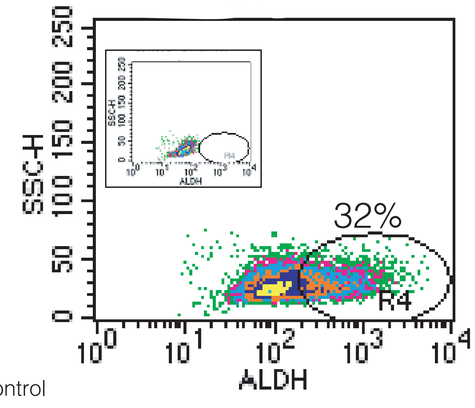


ALDEFLUOR® staining of ALDH^{bright} cell line is stronger at cell concentrations: <1 x 10^6 cell/mL.
Optimal cell concentration may be different for different cell types.

ALDEFLUOR® assay detects hematopoietic cell expansion in CB CD34+/MSC co-cultures

Cord blood (CB) CD34+ cells isolated using EasySep® cell separation, were cultured for 7 days in cytokine-supplemented StemSpan® SFEM alone or in the presence of adherent BM-derived mesenchymal cells (MSCs). MSCs (passage 1) were generated using MesenCult®-XF mesenchymal stem cell medium. ALDH-bright cells (Fig 5) were detected in cultured cells and numbers compared to input CB CD34+ cells numbers (Fig 6). Hematopoietic colony forming cells (CFCs) using MethoCult® H4034 semisolid medium were measured on input CB CD34+ cells and cultured cells (Fig 7).

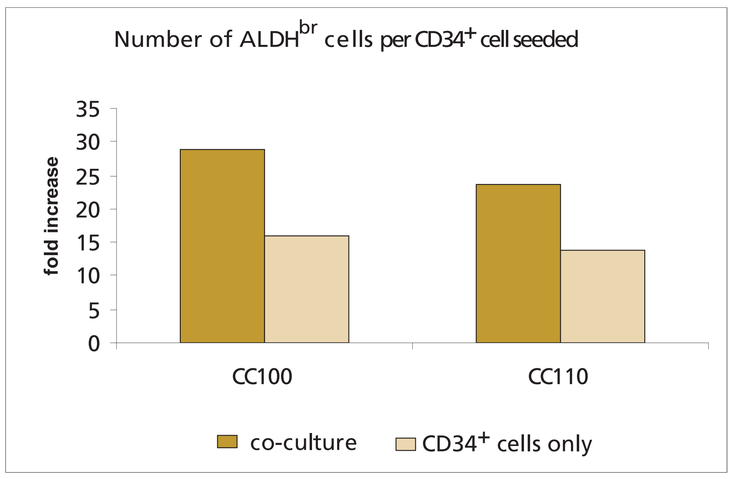
Figure 5: Example of flow cytometry results from ALDEFLUOR® assay on cultured CD34+ CB cells



Inset: DEAB control

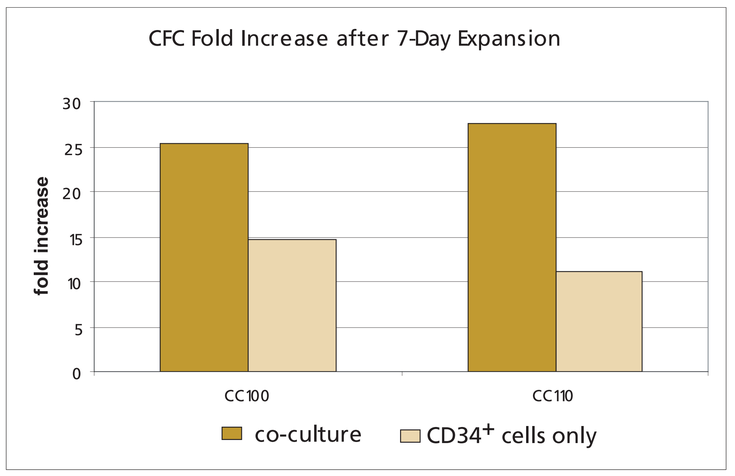
The fold-expansion of ALDH-bright cells (Fig. 6) correlated with the expansion of CFCs (Fig. 7), showing that detection of ALDH-bright cells may be a useful tool for evaluating functional hematopoietic progenitor cells in culture.

Figure 6: Cell expansion results of ALDEFLUOR® assay after 7-day expansion of CD34+ CB cells



CC100 = SCF, FLT3L, IL-3, IL-6
CC110 = SCF, FLT3L, TPO

Figure 7: Colony assay after 7-day expansion of CD34+ CB cells



Conclusions

- ALDEFLUOR® assay detects cells with high ALDH activity (ALDH-bright), a marker for normal and tumor stem cells and progenitors in a variety of tissues (hematopoietic, mesenchymal, endothelial, muscle, intestinal epithelial, mammary, and likely many more).
- The cell membrane must be intact (viable cells). Necrotic or apoptotic cells are not detected.
- Standard protocols, established for human hematopoietic cells, may need to be optimized for other cell types and for cultured cells.
- Functional characterization of ALDH^{bright} and ALDH^{low} subsets is essential to determine whether ALDH activity identifies stem cells in each individual cell type.