

TECHNICAL BULLETIN

A GUIDE TO IDENTIFICATION OF STEM CELLS WITHOUT ANTIBODIES

IDENTIFY STEM CELLS IN TUMORS AND NON-HEMATOPOIETIC TISSUES WITHOUT ANTIBODIES USING ALDEHYDE DEHYDROGENASE EXPRESSION DETECTED BY ALDEFLUOR®

ALDEHYDE DEHYDROGENASE EXPRESSION AND STEM CELLS

Primitive hematopoietic stem cells are relatively resistant to alkylating agents, such as the active derivatives of cyclophosphamide, and this resistance is due to expression of the enzyme aldehyde dehydrogenase (ALDH).¹⁻³ The ALDEFLUOR® fluorescent reagent system uses this increased expression of ALDH in stem cells as a non-immunological method to distinguish stem/progenitor cells from more mature cells by flow cytometry. ALDEFLUOR® was originally developed as a reagent for identification of hematopoietic stem/progenitor cells from human bone marrow, peripheral blood, apheresis collections and umbilical cord blood.⁴⁻⁷ However, ALDEFLUOR® has also proven useful for the identification and characterization of a broad spectrum of non-hematopoietic stem and progenitor cells as described below.

ASSAY PRINCIPLES

The enzyme ALDH converts the fluorescent ALDH substrate, BAAA (BODIPY®-aminoacetaldehyde) into the fluorescent product BAA (BODIPY®-aminoacetate), which is retained inside the cells due to its net negative charge, which disallows passive diffusion. Only viable cells with an intact cellular membrane are capable of retaining the ALDEFLUOR® reaction product. Viable cells expressing high levels of ALDH become brightly fluorescent (ALDH^{br}) and can be identified and enumerated using a standard flow cytometer, or isolated by cell sorting for further purification and characterization.

PROCEDURE SUMMARY

- Uncharged ALDH substrate (BAAA) is taken up by living cells through passive diffusion.
- BAAA is converted by intracellular ALDH into a negatively charged reaction product (BAA), which is retained inside the cells. Those cells that express high levels of ALDH become brightly fluorescent.
- Efflux of BAA by ATP-binding cassette (ABC) transporters is blocked by an inhibitor present in the assay buffer.
- Brightly fluorescent ALDH-expressing cells (ALDH^{br}) are detected in the green fluorescence channel (FL1; 520-540 nm) of a standard flow cytometer.
- Only cells with an intact cellular membrane can retain the ALDEFLUOR® reaction product and therefore only viable cells are identified as ALDH^{br}.
- Diethylaminobenzaldehyde (DEAB), an inhibitor of ALDH activity, is used as a negative control.

WHY USE ALDEFLUOR®?

NO ANTIBODIES. ALDEFLUOR® can be useful when surface markers defining stem or progenitor cells are not known or agreed upon, as staining with antibodies is not required.

EASY. The process is simple and highly reproducible. Primitive cells express more ALDH and will therefore accumulate more fluorescent product (ALDH^{br}).

FLEXIBLE. Works with cryopreserved or fresh samples, as long as they are viable and not fixed.

UTILITY OF ALDH EXPRESSION FOR IDENTIFICATION OF NEURAL, ENDOTHELIAL, MAMMARY, MESENCHYMAL, AND CANCER STEM CELLS

The close association between high ALDH activity and stem and progenitor cell activity is not restricted to the human hematopoietic lineage. Aldehyde dehydrogenase is highly expressed in viable stem and progenitor cells of various other lineages, including endothelial, mesenchymal and neural, and ALDH^{br} human cord blood cells engraft a variety of non-hematopoietic tissues in immunodeficient mice.⁴⁻¹⁴

To date, ALDEFLUOR® has been reported to identify **neural** progenitor cells in mouse,^{11,12} rat¹⁰ and human,¹⁵ as well as primitive progenitor cells in mouse **bone marrow**^{8,9} and even **tunicates**.¹⁶ In humans, ALDEFLUOR® has identified **endothelial**,⁷ **mesenchymal**,⁷ and normal and malignant **mammary**¹⁷ progenitors, as well as **pancreatic**,¹⁸ **leukemic**¹⁹ and **lung cancer** cells,²⁰ and has recognized ALDH overexpression in **squamous cell carcinoma** samples²¹ and cell lines. The diverse utility of ALDEFLUOR® suggests that ALDH activity has been conserved in stem and progenitor cells across a broad spectrum of cell lineages and species.

TECHNICAL TIPS WHEN USING ALDEFLUOR® WITH CELLS OTHER THAN HUMAN HEMATOPOIETIC CELLS

The ALDEFLUOR® reagent is known to act as a substrate for the 1A1 isoform of human ALDH and likely also interacts with isoform 3A1. The interaction of the ALDEFLUOR® reagent with other isoforms of human ALDH has not been determined. Most tissue types and species express some isoform(s) of ALDH, but these enzymes vary in tissue distribution and preferred substrate. Therefore, the ALDEFLUOR® assay may not work with some species and tissue types.

The ALDEFLUOR® kit includes DEAB for use in the assay buffer to inhibit the reaction of ALDH with the ALDEFLUOR® reagent, providing a negative control for the assay. DEAB is known to inhibit the 1A1 isoform of human ALDH. While DEAB has proven to be useful for inhibiting the ALDEFLUOR® reaction in a variety of tissue types and species, it may not be effective in tissues and species expressing other ALDH isoforms. However, in all studies to date, the assay has been performed using DEAB as the inhibitor.

USES STANDARD LAB EQUIPMENT. The reaction causes viable cells to generate a fluorescent product, which can be detected on a standard flow cytometer in the FL1 channel.

INFORMATIVE. Identifies only viable cells with an intact cellular membrane. Expression of cell surface markers can be detected simultaneously, as cells can be counterstained with standard fluorescently-labeled antibodies.



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29140 | Version 1.0.1 | April 2008

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OVERCOMING TECHNICAL ISSUES WITH ALDH INHIBITOR

As noted, the specific ALDH isoform expressed in the cell type being investigated may not be inhibited by DEAB. A lack of difference between test and negative control samples may indicate that the inhibitor was not effective. If DEAB is not effective, ALDH inhibition may be achieved by using the following modifications:

- Use DEAB at 10-fold excess of activated ALDEFLUOR® reagent, on a molar basis.
- Maintain molar ratio of DEAB to activated ALDEFLUOR® reagent when titrating ALDEFLUOR® substrate.
- Other ALDH inhibitors can be used as appropriate for the enzyme expressed (e.g. disulfuram inhibits several mammalian ALDH gene products).
- Studying reaction kinetics (i.e. a progressive increase in ALDEFLUOR® fluorescence with time of reaction) could reveal DEAB-insensitive ALDH activity in cells.

OVERCOMING "MASKING" OF ALDH ACTIVITY IN MOUSE SAMPLES

In mouse bone marrow samples it may be difficult to identify ALDH^{br} stem/progenitor cells. The ALDH^{br} stem/progenitor cell population may be masked by the high level of ALDH activity in myeloid cells,²² which leads to high levels of ALDEFLUOR® staining in these cells. Identification and isolation of ALDH^{br} cells from heterogeneous cell suspensions can be facilitated by removing the myeloid cells and other mature cells that have high ALDH activity or strong background fluorescence, thereby enriching the sample for primitive cells prior to performing the ALDEFLUOR® assay. We recommend the immunomagnetic enrichment of primitive cells by removing mature cells with a combination of antibodies to lineage-specific markers and magnetic beads. We can suggest the appropriate cell separation kit if you email techsupport@stemcell.com with the cell type(s) you are looking to remove, the species your cells are sourced from, and the cell type(s) you are looking to isolate.

OTHER TIPS

- Ice is the universal efflux inhibitor. Keep all ALDEFLUOR®-reacted samples on ice.
- With cell lines it is helpful to use gating strategies that exclude dead cells by using a viability marker (e.g. propidium iodide or 7-AAD) before gating on green fluorescence for ALDH^{br} cells.
- The Side Population assay, for identification of the stem cell population in cell sources such as hematopoietic tissues, can be used in conjunction with the ALDEFLUOR® assay. It is recommended that the cells be labeled with Hoechst dye first and then with ALDEFLUOR®, which can be followed by immunophenotyping.²²

ADDITIONAL TECHNICAL BULLETINS AVAILABLE

Technical bulletins are available to assist with immunophenotyping of ALDEFLUOR®-treated cells and for compensation setting of flow cytometers. The ALDEFLUOR® Technical Bulletins are available at <http://www.stemcell.com/technical/aldh.aspx> and include:

- Immunophenotyping (http://www.stemcell.com/technical/ALDH_immunophenotyping.pdf) and
- Compensation (http://www.stemcell.com/technical/ALDH_compensation.pdf)

For additional support, contact STEMCELL Technologies' Technical Support department at techsupport@stemcell.com.

PRODUCT INFORMATION

PRODUCT DESCRIPTION	QUANTITY	CATALOG #
ALDEFLUOR®	40 tests	01700

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