ALDEFLUOR™ Kit

For the Identification, Evaluation and Isolation of Stem and Progenitor Cells Expressing High Levels of ALDH

Catalog #01700 1 Kit

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Product Description

ALDEFLUOR™ is a reagent kit that is used to identify human cells that express high levels of the enzyme aldehyde dehydrogenase (ALDH). The activated ALDEFLUOR™ Reagent, BODIPY-aminoacetaldehyde (BAAA), is a fluorescent non-toxic substrate for ALDH, which freely diffuses into intact and viable cells. In the presence of ALDH, BAAA is converted into BODIPY-aminoacate (BAA), which is retained inside the cells. The amount of fluorescent reaction product is proportional to the ALDH activity in the cells and is measured using a flow cytometer. Viable ALDH-bright ($ALDH^{br}$) cells can, in principle, be isolated using a cell sorter. Active efflux of the reaction product is inhibited by an efflux inhibitor in the ALDEFLUOR™ Assay Buffer. A specific inhibitor of ALDH, diethylaminobenzaldehyde (DEAB), is used to control for background fluorescence.

ALDEFLUOR™ is optimized for the detection of ALDHbr hematopoietic cells in human blood and bone marrow, but it can also be used with non-hematopoietic cells. For a full list of ALDEFLUOR™ products, please visit our website at www.stemcell.com.

Product Information

The following components are sold as part of the ALDEFLUOR™ Kit (Catalog #01700) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
Dry ALDEFLUOR™ Reagent*	01703	50 µg	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
ALDEFLUOR™ Diethylaminobenzaldehyde (DEAB) Reagent, 1.5 mM in 95% ethanol**	01705	1 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
Hydrochloric Acid (HCl, 2 N)**	01704	1.5 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
Dimethylsulphoxide (DMSO)**	01706	1.5 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
ALDEFLUOR™ Assay Buffer**	01701	4 x 25 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.

*ALDEFLUOR™ Reagent is not cytotoxic. The combination of the dry ALDEFLUOR™ Reagent, DMSO and HCl shows no cytotoxic or phototoxic effects at concentrations 100-fold above those used in this assay.
**Please refer to the Safety Data Sheet for hazard information.

Directions for Use

Please read the entire protocol before proceeding.

A) ALDEFLUOR™ ACTIVATION

The dry ALDEFLUOR™ Reagent is provided in a stable, inactive form (BODIPY-aminoacetaldehyde-diethyl acetate, BAAA-DA). For use, the dry ALDEFLUOR™ Reagent is dissolved in DMSO, converted to the fluorescent-activated ALDEFLUOR™ Reagent (BAAA) by treatment with 2 N HCl and diluted with ALDEFLUOR™ Assay Buffer:

1. Assemble all necessary supplies and allow kit reagents to come to room temperature (15 - 25°C) before use.
2. Add 25 µL of DMSO to the vial of dry ALDEFLUOR™ Reagent, mix well and let it stand for 1 minute at room temperature (15 - 25°C).
   NOTE: The dry ALDEFLUOR™ Reagent is an orange-red powder that changes to a bright yellow-green color upon addition of DMSO.
3. Add 25 µL of 2 N HCl and mix well. Incubate this mixture for 15 minutes at room temperature (15 - 25°C).
   NOTE: Adding 2 N HCl before DMSO will render the product inactive.

4. Add 360 µL of ALDEFLUOR™ Assay Buffer to the vial and mix.

   NOTE: Upon addition of the ALDEFLUOR™ Assay Buffer, the solution may appear slightly cloudy. This does not affect the assay performance.
5. Keep the activated ALDEFLUOR™ Reagent at 2 - 8°C during use.
6. Aliquot the remaining activated ALDEFLUOR™ Reagent and store at -20°C.

B) CELL SAMPLE PREPARATION

1. Prepare fresh or previously frozen cell samples according to standard procedures for the cell type.
2. If using blood samples where the red blood cell (RBC) to leukocyte ratio (RBC:WBC) of the specimen is > 2:1, lyse the erythrocytes with Ammonium Chloride Solution (Catalog #07800).
3. After RBC lysis, centrifuge the sample for 5 minutes at 250 x *g*. Remove the supernatant and suspend cells in 1 mL of ALDEFLUOR™ Assay Buffer.
4. Perform a cell count.
5. If using hematopoietic cells (e.g. peripheral blood, apheresis product, bone marrow or cord blood) adjust the sample to a concentration of 1 x 10^6 cells/mL with the ALDEFLUOR™ Assay Buffer.

   NOTE: For other cell types, different cell concentrations may be more appropriate. For optimization of ALDEFLUOR™ staining conditions for non-hematopoietic cells, refer to the Technical Bulletin: ALDEFLUOR™ Assay Optimization (Document #29902), available on our website at www.stemcell.com.

C) ALDEFLUOR™ ASSAY

1. Label one “test” and one “control” tube for each sample to be tested. Place 1.0 mL of the adjusted cell suspension (section B) into each “test” sample tube.
2. Add 5 µL of ALDEFLUOR™ DEAB Reagent to the “control” tube. Recap control tube and DEAB vial immediately.

   NOTE: ALDEFLUOR™ DEAB is provided in 95% ethanol. Recap immediately to prevent evaporation.
3. Add 5 µL of the activated ALDEFLUOR™ Reagent per milliliter of sample to the first sample “test” tube. Mix and immediately transfer 0.5 mL of the mixture to the 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.
4. Add control and substrate solutions as described in steps 2 and 3 above for each sample to be tested.
5. Incubate “test” and “control” samples for 30 to 60 minutes at 37°C (do not exceed 60 minutes).

   NOTE: Optimal incubation times may vary between different cell types. For suggestions on optimization of ALDEFLUOR™ staining conditions for non-hematopoietic cells, cultured cells, and cell lines, refer to the Technical Bulletin: ALDEFLUOR™ Assay Optimization (Document #29902), available on our website at www.stemcell.com.
6. Following incubation, centrifuge all tubes for 5 minutes at 250 x *g* and remove supernatant. Resuspend cell pellets in 0.5 mL of ALDEFLUOR™ Assay Buffer and store the cells on ice or at 2 - 8°C.

   NOTE: If immunophenotyping is to be performed, add and incubate the antibodies after step 6. To prevent efflux of the ALDEFLUOR™ product it is important that the antibody incubation is performed in ALDEFLUOR™ Assay Buffer. Whenever possible keep the cells chilled (2 - 8°C or on ice) to slow down the product efflux.
7. Optional: Perform a viability cell count. If the sample contains fewer than 90% viable cells, it is recommended to stain cells with a DNA dye such as propidium iodide or 7-actinoaminomycin-D in order to stain dead and late apoptotic cells.

D) FLOW CYTOMETER SET-UP AND DATA ACQUISITION

Refer to the Technical Bulletin: The Basic FACS on ALDEFLUOR™ (Document #28000), available on our website at www.stemcell.com.

Notes and Tips

- Fresh or previously frozen samples can be analyzed for $ALDH^{br}$ cells. However, the ALDEFLUOR™ kit will only detect ALDH activity in cells that are viable and have intact cell membranes.
- Removal of erythrocytes from the sample is required. Erythrocytes may be removed by lysis using reagents that do not contain detergents or fixatives (e.g. Ammonium Chloride Solution, Catalog #07800). They may also be removed by density centrifugation.
- When frozen aliquots of the activated ALDEFLUOR™ Reagent are thawed, a small precipitate (pellet) may be observed. Before use, mix the thawed reagent to resuspend the precipitate. This precipitate does not affect assay performance.

- The cell lines A549 (lung carcinoma), SKBR3 (breast cancer) and K562 (CML) express ALDH activity and can be used as positive controls for the ALDEFLUOR™ assay. In addition, commercially-available bone marrow mononuclear cells (Catalog #ABM007F, ABM010F) can also be used as positive controls.
- Identification of rare $ALDH^{br}$ cells in heterogeneous cell samples can be improved by removing mature hematopoietic cells on the basis of lineage antigen expression and enriching for $ALDH^{br}$ cells by using the RosetteSep™ Human Progenitor Cell Enrichment Kit or EasySep™ Human CD34 Positive Selection Kit prior to performing the ALDEFLUOR™ assay.

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