AldeGreen™ ALDH Detection Assay

Stem Cell Assay

Cat. # SCR151

pack size: 1 Kit

Store at 2-8ºC

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Data Sheet

Description

The AldeGreen™ ALDH Detection Assay enables flow cytometry-based detection of intracellular Aldehyde Dehydrogenase (ALDH) enzyme activity. High ALDH activity serves as a universal marker of stem cells, both normal and malignant.

The assay employs a fluorescent and non-toxic ALDH substrate (AldeGreen™) that diffuses freely into intact and viable cells, but remains trapped inside the cells once converted by ALDH into the corresponding acid. The amount of fluorescence produced is proportional to the ALDH activity in the cells and is measured by flow cytometry, allowing fluorescence-activated cell sorting (FACS). This kit supplies the ALDH inhibitor diethylaminobenzaldehyde (DEAB), which is used in negative control testing necessary for background fluorescence assessment.

Kit Components

1. AldeGreen™ Reagent: (Part No. CS226325). One (1) vial containing 50 µg of AldeGreen™ reagent powder. Store at 2-8°C.
2. DEAB Reagent: (Part No. CS216595). One (1) vial containing 1 mL of DEAB (Diethylaminobenzaldehyde) reagent. Store at 2-8°C.
3. Hydrochloric Acid (2N): (Part No. CS216594). One (1) vial containing 1.5 mL of 2N hydrochloric acid. Store at 2-8°C.
4. Dimethylsulphoxide: (Part No. CS216593). One (1) vial containing 1.5 mL of Dimethylsulphoxide (DMSO). Store at 2-8°C.
5. AldeGreen™ Assay Buffer: (Part No. CS226324). Four (4) bottles containing 25 mL of AldeGreen™ Assay Buffer each. Store at 2-8°C.
6. Verapamil: (Part No. CS220012). Four (4) vials containing 615 µg Verapamil powder each. Store at 2-8°C.

Storage and Stability

- Store kit at 2-8°C.
- Reconstituted and activated AldeGreen™ Reagent may be stored in aliquots at -20°C protected from light for up to 1 year. Repeated freezing and thawing is not recommended.
- AldeGreen™ Assay Buffer supplemented with Verapamil may be stored at 2-8°C in aliquots for up to 3 months.

Warning and Precautions

Handle all reagents using aseptic techniques.

DMSO, DEAB and HCl are irritants to skin, eyes and airways. Refer to MSDS documents for more information.

Quality Control Testing

- Each lot is tested by measuring ALDH activity in SK-BR-3 cells by flow cytometry.
- AldeGreen™ Reagent is 95% pure (PMR)

Ver 2.0/2019-05-30/SCR151/NJA

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

Representative Data

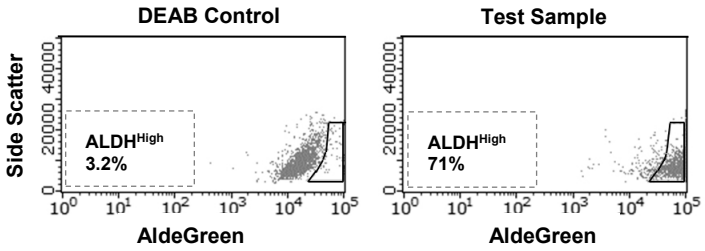

Figure 1. ALDH activity in SKBR-3 breast cancer cells.

AldeGreen™ reaction was carried out according to the standard protocol using10^5 cells and a 45-min incubation time at 37°C. The gated area corresponds to AldeGreen™^{High} population.

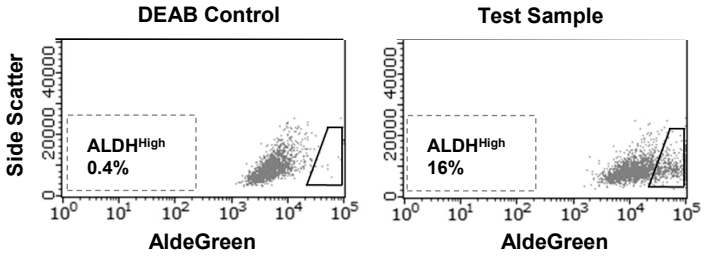

Figure 2. ALDH activity in UM-SCC-47 head and neck squamous cell carcinoma cell line (Cat. No. SCC071).

AldeGreen™ reaction was carried out according to the standard protocol using 10^5 cells and 45-min incubation time at 37°C. The gated area corresponds to AldeGreen™^{High} population.

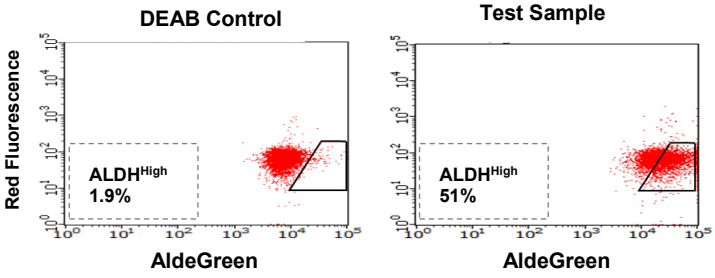

Figure 3. ALDH activity in K562 chronic myeloid leukemia cells.

AldeGreen™ reaction was carried out according to the standard protocol using 10^5 cells and 45-min incubation time at 37°C. The gated area corresponds to AldeGreen™^{High} population.

Important Notes before Starting

- AldeGreen™-positive cells may be imaged using a fluorescence microscope or quantified using a flow cytometer. A 488 nm argon ion laser may be used for excitation and an optical filter set to detect 515-545 nm fluorescence.
- All live intact cells (even with low ALDH expression) become fluorescent upon exposure to activated AldeGreen™. The generation of a DEAB-treated control is necessary for each cell sample to establish baseline fluorescence.
- Samples containing Red Blood Cells (RBC) require lysis with ammonium chloride-based buffered solution (Minn, I., *et al.* (2014). Nat. Commun. 5:3662).
- To achieve the optimal signal to noise ratio for your cell type(s) of interest, the cell concentration may require adjustment. Prepare a range of cell concentrations when performing the initial AldeGreen™ assay. A DEAB-treated control must be run in parallel with each cell concentration.
- The optimal incubation time with activated AldeGreen™ Reagent may need to be determined emprically for each cell type. Do not exceed 60 minutes.
- AldeGreen™ Assay buffer is supplemented with the efflux inhibitor Verapamil to prevent efflux of AldeGreen™ reaction product and loss of fluorescence. Alternative efflux inhibitors can be tested with this assay to optimize detection of AldeGreen™ reaction product. It is also important to keep the cells on ice after completion of the reaction to prevent efflux and loss of fluorescence.
- Higher DEAB concentration may be required for cell types with very high ALDH activity.
- SK-BR-3 (breast cancer) and K562 (CML) cells have high ALDH activity and can be used as positive controls for the AldeGreen™ Assay.

Protocols

A. Reagent Preparation

AldeGreen™ Assay Buffer Preparation

1. Remove 1 mL of AldeGreen™ Assay Buffer from one of the four bottles supplied with the kit and add it to one of four vials containing Verapamil powder. Mix well by vortexing the vial for 2-3 min and transfer the solution back to the same bottle of AldeGreen™ Assay Buffer. Label the bottle accordingly.

   Important note: *AldeGreen™ Assay buffer needs to be supplemented with the efflux inhibitor Verapamil to prevent efflux of AldeGreen™ reaction product and loss of fluorescence.*

   Note: *AldeGreen™ Assay Buffer supplemented with Verapamil may be stored at 2-8°C for up to 3 months.*

AldeGreen™ Generation

2. Allow all reagents to equilibrate to room temperature.
3. Add 25 µL of DMSO to the vial of dry AldeGreen™ Reagent and mix well. Expect the orange-red powder to turn into bright yellow-green color solution upon addition of DMSO.
4. Add 25 µL of 2N HCl and mix well. Incubate the mixture for 15 min at room temperature.
5. Add 360 µL of AldeGreen™ Assay Buffer supplemented with Verapamil to the vial and mix.
6. Keep the AldeGreen™ at 2-8°C during use.

   Note: *Aliquot the remaining AldeGreen™ and store at -20°C in the dark. Avoid repeated freeze thaw cycles.*

B. Cell Sample Preparation

1. Depending on the cell type you are working with (adherent, suspension, fresh or frozen) follow a standard procedure to suspend cells in growth media and count. Adjust cell number, pellet the cells and replace growth media with 1 mL of AldeGreen™ Assay Buffer supplemented with Verapamil.

   Note: *Cell concentration may require optimization for each cell type (e.g. optimal concentration of SK-BR-3 cells is 1-2x10^5 cells/mL, see Fig. 2). Testing a range of cell concentrations is recommended for determining the highest signal to background ratio (i.e. Test sample to DEAB-treated control sample).*

C. AldeGreen™ Assay

1. Label one “test” and one “control” 1.5 mL microcentrifuge tube for each cell sample to be tested. Transfer 1 mL of the cell suspension from Step B to the corresponding “test” tube.
2. Add 5 µL of DEAB reagent to each “control” tube. Recap “control” tube and DEAB vial to prevent ethanol evaporation.
3. Add 5 µL of the prepared AldeGreen™ from Step A.5 to the “test” tube that contains 1 mL of cell suspension. Mix and *immediately* transfer 0.5 mL of the mixture to the “control” tube containing DEAB.
4. Repeat Steps C.2-3 for each sample to be tested.
5. Incubate “test” and “control” tubes at 37°C for 30-60 minutes. Exact incubation time may require optimization for different cell types.
6. Centrifuge all tubes at 250 x g for 5 minutes and discard supernatant. Resuspend cell pellets in 0.5 mL of AldeGreen™ Assay Buffer supplemented with Verapamil and keep the cells on ice to prevent ALDH reaction product efflux.
7. **Optional:** Immunophenotyping of intact cells using fluorescent antibodies against cell surface markers may be performed at this point while maintaining the cells in AldeGreen™ Assay Buffer supplemented with Verapamil on ice to prevent efflux of AldeGreen™ reaction product.

D. Data Acquisition

1. Depending on the make and model of the flow cytometer, the workflow may vary. In set-up mode, adjust FSC and SSC voltages and gains using DEAB “control” sample and create region (R1) to encompass the nucleated cells.
2. Create AldeGreen™ vs. SSC dot plot gated on R1 and adjust voltage so the right edge of the DEAB “control” sample cell population is placed at the second decade on X-axis.
3. Run a corresponding “test” sample and create a region R2 that contains ALDH^{High} cells (see Fig. 2 and Fig. 3).
4. Switch the flow cytometer to sample acquisition mode and collect data for all “control” and “test” samples.