

Data Sheet

AldeRed® ALDH Detection Assay

Stem Cell Assay

SCR150**Pack size: 1 kit****Store at 2-8 °C**

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Background

The AldeRed® 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 AldeRed® Reagent supplied corresponds to AldeRed® 588 aldehyde diethyl acetal, which needs to be converted by acid hydrolysis to generate the red-shifted fluorescent substrate AldeRed® 588 aldehyde (AldeRed® 588-A) for ALDH. AldeRed® 588-A can be successfully utilized in the presence of green fluorophores, including cells expressing green fluorescent proteins.

Assay Principle

The assay employs a fluorescent and non-toxic ALDH substrate (AldeRed® 588-A) 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 diethylamino benzaldehyde (DEAB), which is used in negative control testing necessary for background fluorescence assessment.

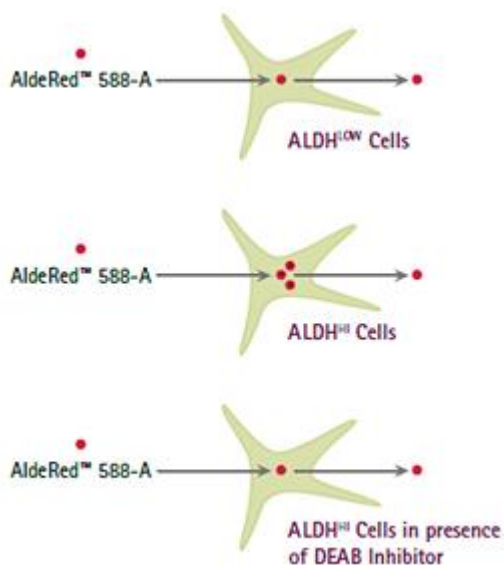


Figure 1. AldeRed® 588-A contains an aldehyde moiety that can serve as a substrate for ALDH. AldeRed® 588-A freely diffuses into cells and becomes trapped in the cytoplasm after the conversion of the aldehyde into the corresponding acid by ALDH. The accumulation of the oxidized AldeRed® 588-A causes the subset of ALDH^{High} cells to become highly fluorescent. ALDH^{High} cells are identified by comparison to baseline fluorescence from DEAB-treated cells.

Quality Control Testing

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

Storage and Stability

See [Materials Provided](#) for storage.

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

Warnings 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.

Materials Provided

- AldeRed® Reagent (CS216596): One (1) vial containing 50 µg of AldeRed® reagent powder.
Store at 2-8 °C.
- DEAB Reagent (CS216595): One (1) vial containing 1 mL of DEAB (Diethylaminobenzaldehyde) reagent.
Store at 2-8 °C
- Hydrochloric Acid (2 N) (CS216594): One (1) vial containing 1.5 mL of 2 Normal hydrochloric acid.
Store at 2-8 °C.
- Dimethylsulphoxide (CS216593): One (1) vial containing 1.5 mL of Dimethylsulphoxide (DMSO).
Store at 2-8 °C.
- AldeRed® Assay Buffer (CS216592): Four (4) bottles containing 25 mL of AldeRed® Assay Buffer each.
Store at 2-8 °C.
- Verapamil (CS220012): Four (4) vials containing 615 µg Verapamil powder each.
Store at 2-8 °C.

Representative Lot Data

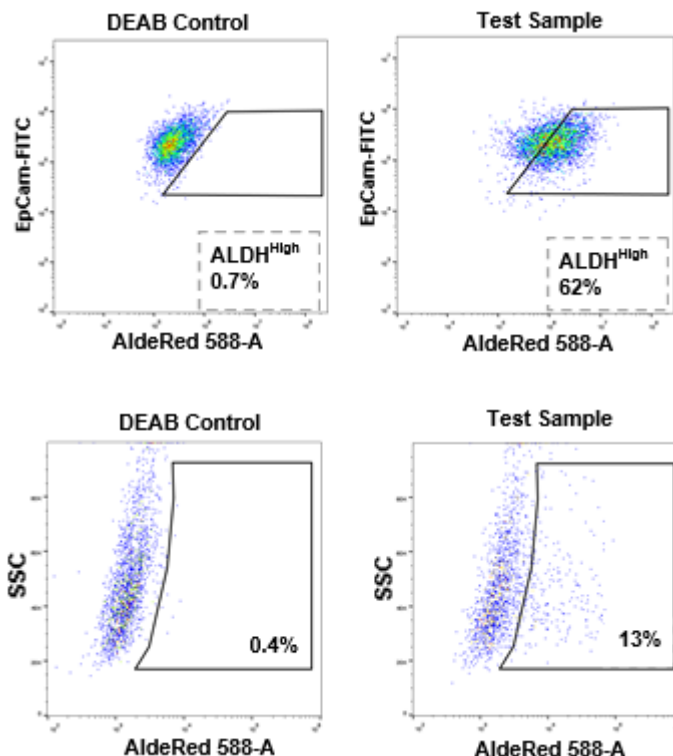


Figure 2. Co-staining of SK-BR-3 cells with AldeRed[®] 588-A and Milli-Mark[®] EpCam-FITC antibody (clone mAB8, FCMAB264F).

AldeRed[®] 588-A reaction was carried according to the standard protocol using 10^5 cells and a 30-min incubation time at 37 °C, followed by a 1-hour staining with EpCam-FITC antibody carried out in AldeRed[®] Assay buffer supplemented with Verapamil at 2-8 °C. The gated area corresponds to EpCam^{High}-AldeRed[®] High population.

Figure 3. ALDH activity in head and neck squamous carcinoma cells (UM-SCC-47, SCC071).

AldeRed[®] 588-A reaction was carried out according to the standard protocol using 10^5 cells and a 30-minute incubation time at 37 °C. A combination of green laser (532 nm) as the excitation and PE-Texas Red wavelength (615 nm) detector channel was used for the detection of AldeRed[®] 588-A oxidation product.

Protocols

Important Notes before Starting

- For optimal excitation and emission of AldeRed[®] 588-A, use a combination of blue laser (488 nm) or green laser (532 nm) with PE-Texas Red detector (615 nm). Detection in proximal measurement channels (For example, PE) is also possible but may affect delta MFI (mean fluorescence intensity) between Test sample and DEAB-treated control sample.
- All live intact cells (even with low ALDH expression) become fluorescent upon exposure to activated AldeRed[®] 588-A. 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.²
- 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 AldeRed[®] assay. A DEAB-treated control must be run in parallel with each cell concentration.
- The optimal incubation time with activated AldeRed[®] Reagent may need to be determined empirically for each cell type. Do not exceed 60 minutes.
- AldeRed[®] Assay buffer is supplemented with the efflux inhibitor Verapamil to prevent efflux of AldeRed[®] 588-A reaction product and loss of fluorescence. Alternative efflux inhibitors can be tested with this assay to optimize detection of AldeRed[®] 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 AldeRed[®] Assay.

Reagent Preparation

AldeRed® Assay Buffer Preparation

Remove 1 mL of AldeRed® 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 AldeRed® Assay Buffer. Label the bottle accordingly.

Note:

- AldeRed® Assay buffer needs to be supplemented with the efflux inhibitor Verapamil to prevent efflux of AldeRed® 588-A reaction product and loss of fluorescence.
- AldeRed® Assay Buffer supplemented with Verapamil may be stored at 2-8 °C for up to 3 months.

AldeRed® 588-A Generation

1. Allow all reagents to equilibrate to room temperature.
2. Add 25 µL of DMSO to the vial of dry AldeRed® Reagent and mix well. Expect violet pellet to turn into red color solution upon addition of DMSO.
3. Add 25 µL of 2 N HCl and mix well. Incubate the mixture for 15 min at room temperature.
4. Add 360 µL of AldeRed® Assay Buffer supplemented with Verapamil to the vial and mix.
5. Keep the AldeRed® 588-A at 2-8 °C during use.

Note: Aliquot the remaining AldeRed® 588-A and store at –20 °C in the dark. Avoid repeated freeze thaw cycles.

Cell Sample Preparation

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 AldeRed® Assay Buffer supplemented with Verapamil.

Note: Cell concentration may require optimization for each cell type (For example, optimal concentration of SK-BR-3 cells is $1-2 \times 10^5$ cells/mL, see Fig. 2). Testing a range of cell concentrations is recommended for determining the highest signal to background ratio (For example, Test sample to DEAB-treated control sample).

AldeRed® 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 [Cell Sample Preparation](#) 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 AldeRed® 588-A from [Reagent Preparation](#) step.4 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 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 AldeRed® Assay Buffer supplemented with Verapamil and keep the cells on ice to prevent ALDH reaction product efflux.

Optional: Immunophenotyping of intact cells using fluorescent antibodies against cell surface markers may be performed at this point while maintaining the cells in AldeRed® Assay Buffer supplemented with Verapamil on ice to prevent efflux of AldeRed® reaction product.

Data Analysis

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 AldeRed® 588-A 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.

References

1. Dolle, L., et al. (2015). Am. J. Physiol. Gastrointest. Liver Physiol. 308(7):G573-G578.
2. Minn, I., et al. (2014). Nat. Commun. 5:3662.

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