

A red-shifted fluorescent substrate for aldehyde dehydrogenase, AldeRed 588-A, for labeling viable ALDH-positive cells



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ABSTRACT

Normal and cancer stem cells can be isolated based upon the enzymatic activity of aldehyde dehydrogenase I (ALDH1), a detoxifying enzyme responsible for oxidation of hazardous aldehyde byproducts. ALDH1 has been used to isolate cancer stem cells from various human malignancies including bladder, breast, cervical, colon, head and neck, liver, lung, pancreas, prostate and ovary. Currently, the ALDEFLUOR™ assay, is the only commercially available reagent for ALDH detection in live cells. The substrate used in this assay primarily emits in the green region of the electromagnetic spectrum (512nm). For researchers with valuable cell and transgenic animal models in which the target gene of interest has been tagged with eGFP, ALDEFLUOR therefore cannot be used. Selection of cells positive for aldehyde dehydrogenase (ALDH) activity from a green fluorescent background is thus difficult with existing reagents. We now describe a red-shifted fluorescent substrate for ALDH, AldeRed 588-A, that provides additional flexibility for utilizing ALDH as a marker for stem cell and cancer stem cell isolation. The activity of AldeRed 588-A was compared with the ALDEFLUOR reagent and demonstrated similar ability to fractionate ALDH^{POS} cells in a number of cell lines tested.

MATERIALS and METHODS

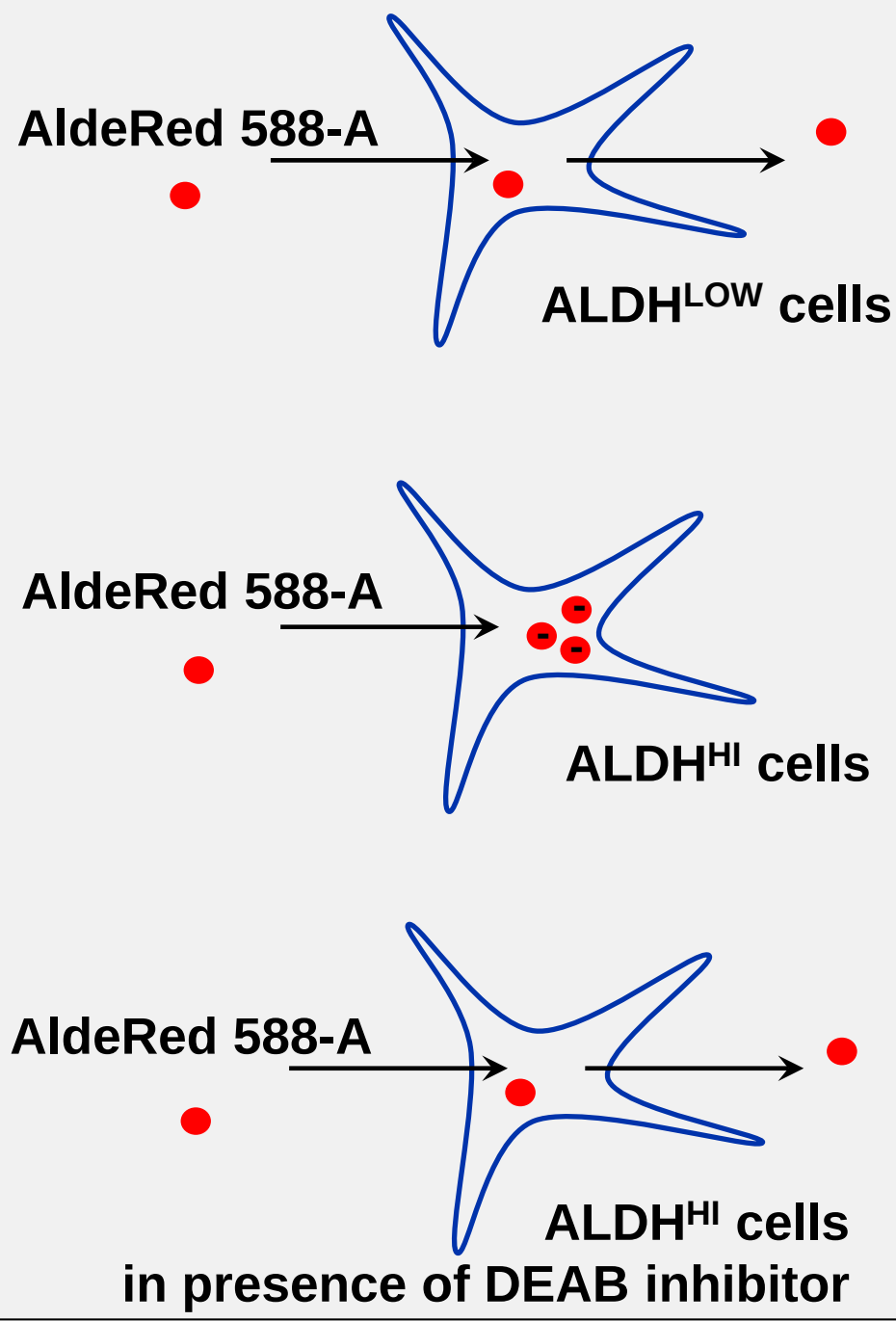
Method principal:

Uncharged ALDH-substrate has the ability to diffuse passively into live cells. Intracellular ALDH enzymes convert the substrate into a negatively charged reaction product, which is trapped inside cells, causing ALDH^{High} cells to become brightly fluorescent.

AldeRed 588-A staining and detection

Dry AldeRed 588-A reagent was resuspended in DMSO and activated by addition of 2N HCl. Each cell sample was suspended in the Assay Buffer and AldeRed 588-A reagent was added. Immediately, the sample was split between two tubes, one of which contained DEAB, an inhibitor of ALDH. Reactions were carried at 37°C for 30 min followed by a round of wash in Assay Buffer.

Detection of AldeRed 588-A was carried on cytometry instruments equipped with blue and/or green laser with PE and/or PE-TexasRed detectors (Guava, Muse, ACEA Novocyte, Beckman Astrios).



Detection of ALDH in SKBR3 cell line using ALDEFLUOR™ kit

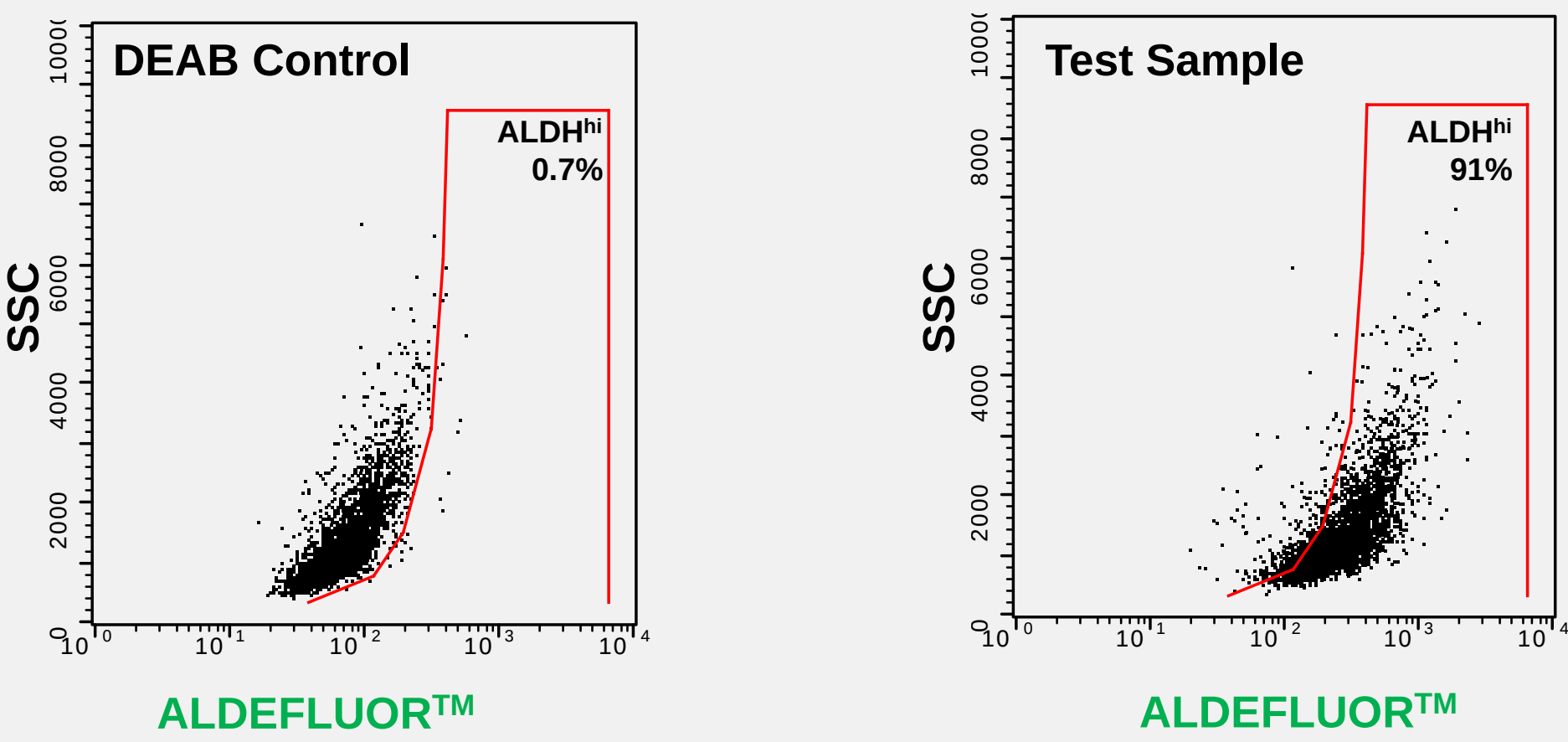


Figure 1. ALDH detection in human adenocarcinoma cells, SKBR3, using ALDEFLUOR™ kit. A combination of Blue laser (488 nm) with FITC detector (525 nm) was used.

CONCLUSIONS

- AldeRed 588-A and ALDEFLUOR™ reagents demonstrate similar ability to fractionate ALDH^{High} cells in a number of cell lines tested.
- Cell staining by AldeRed 588-A is red-shifted and can be combined with the fluorophores emitting in Green spectrum (e.g. FITC, GFP).
- AldeRed 588-A is ideal for cancer and stem cell researchers previously limited to detecting ALDH-positive cells with green fluorescent label.

RESULTS

ALDH detection in SKBR3 cells using AldeRed 588-A

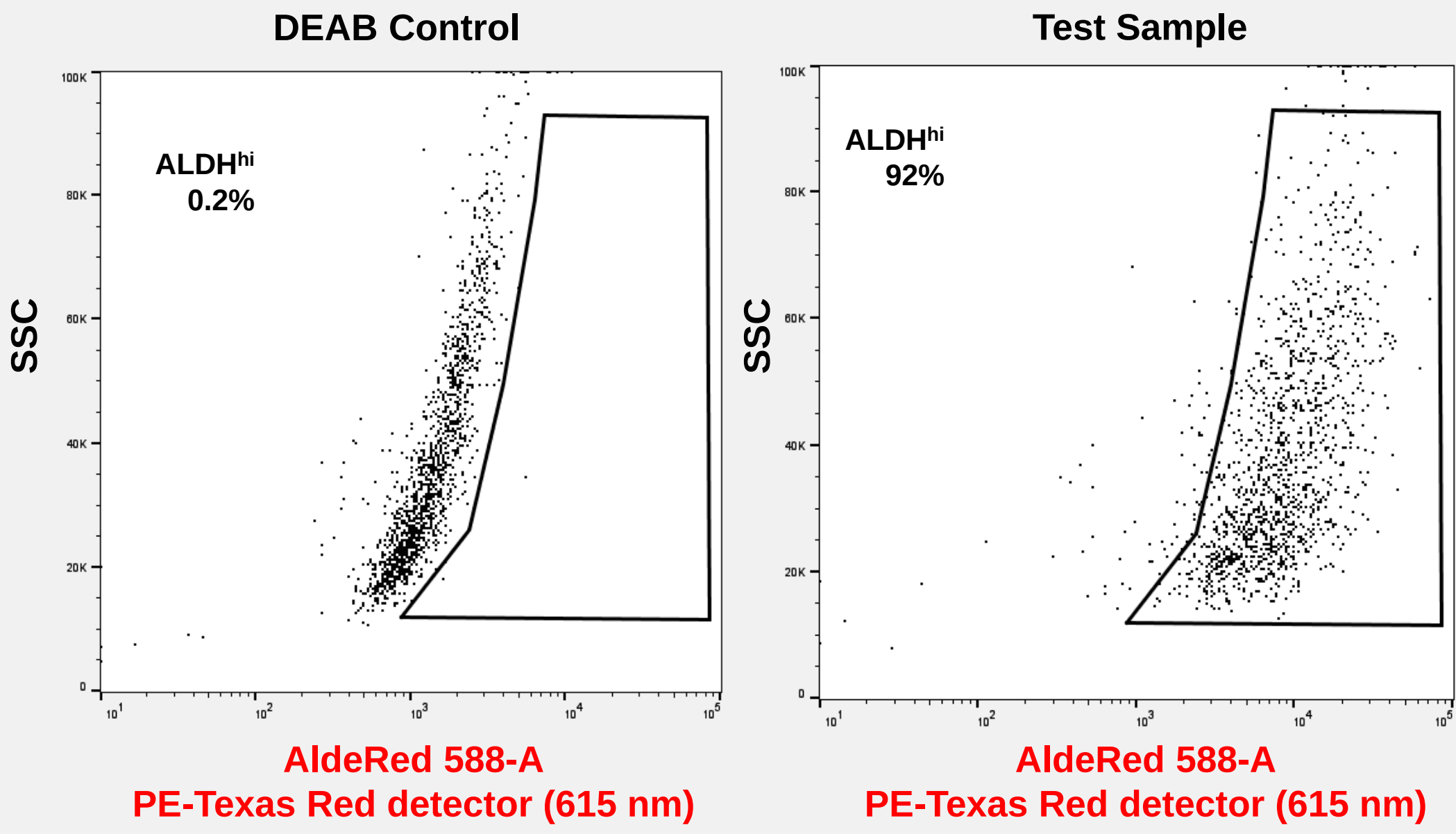


Figure 2. A combination of Green laser (532 nm) with PE-Texas red detector (615 nm)

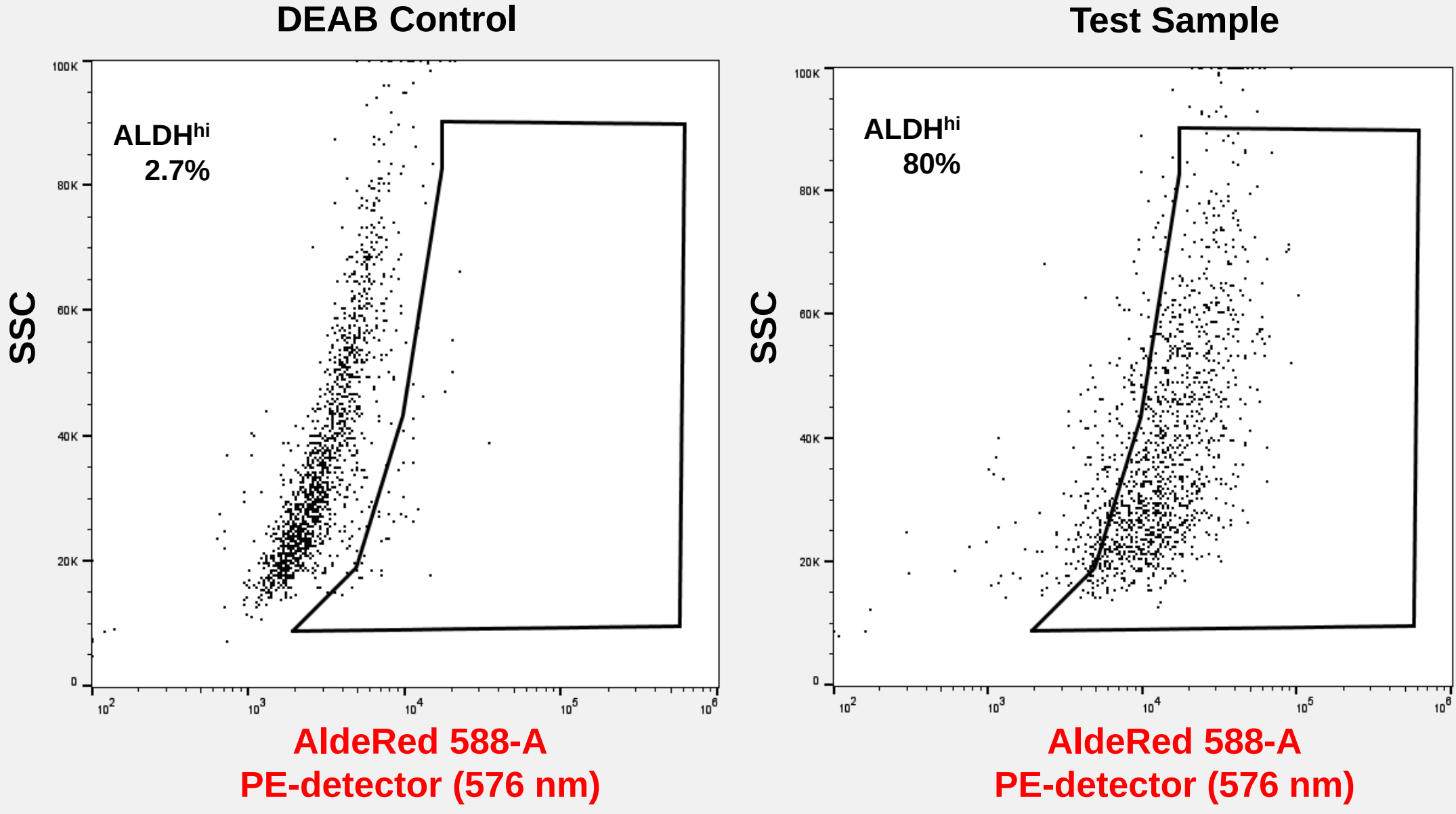


Figure 3. A combination of Blue laser (488 nm) with PE detector (576 nm)

Co-staining of SKBR3 cells with anti-EpCAM-FITC conjugated antibody and AldeRed 588-A

EpCAM, CD326, is involved in tumorigenesis and metastasis of carcinomas, and as such, can act as a potential prognostic marker and as a potential target for immunotherapeutic strategies. Milli-Mark EpCam-FITC antibody, clone mAB8 (Millipore PN FCMA264F) was used in this experiment.

Limitations to ALDEFLUOR™:

- Only detects in green channel
- Can not be used in GFP-positive cells and with FITC labeled antibodies

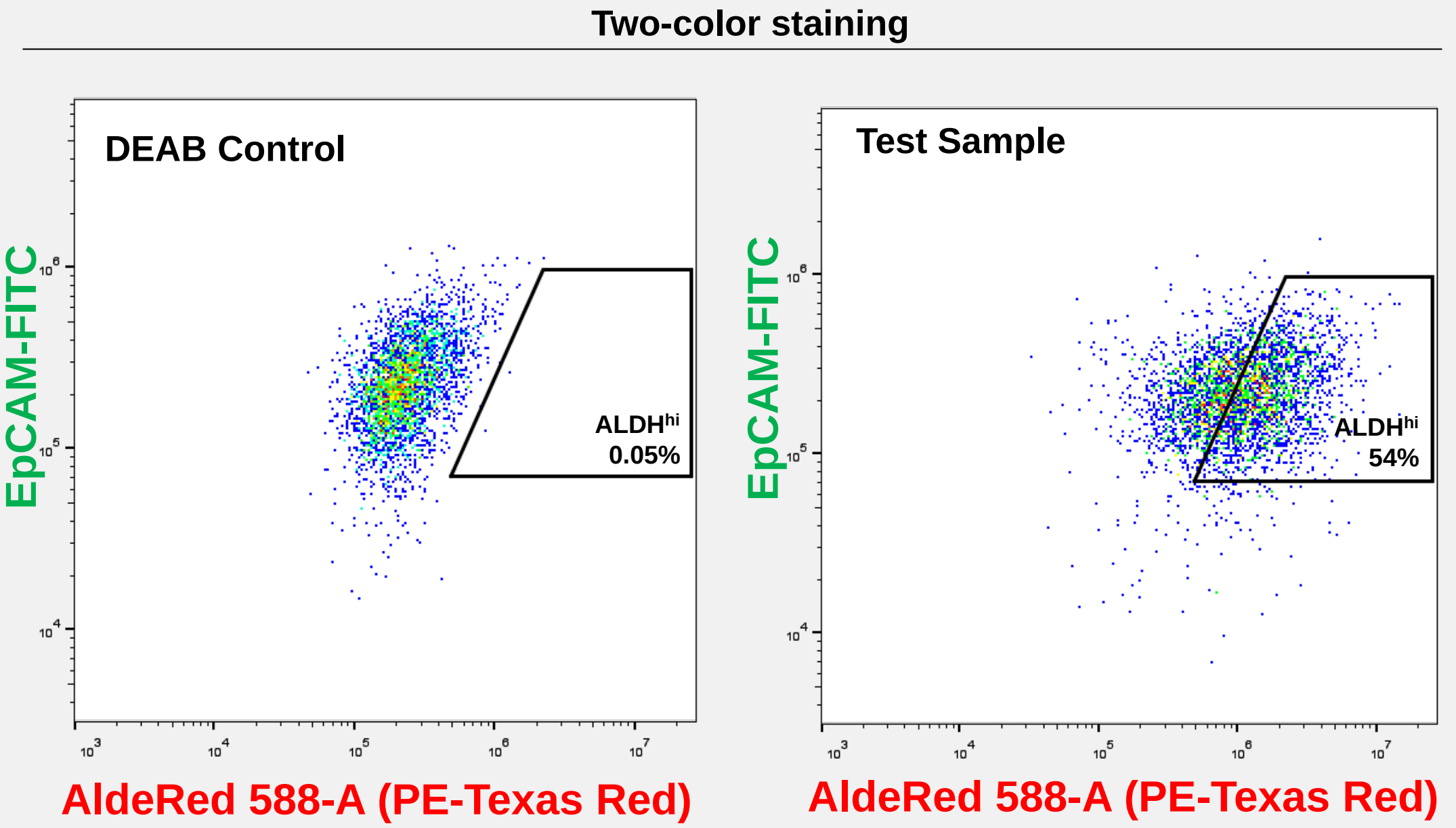


Figure 4. Co-staining of SKBR3 cells using anti-EpCAM-FITC antibody and AldeRed 588-A.

ALDH detection in Head and Neck Squamous Carcinoma cell line

UM-SCC-47 (Millipore PN SCC071) is unique head and neck squamous carcinoma cell line isolated from primary tumor and containing integrated human papillomavirus (HPV-16) and characterized by presence of ALDH+ cancer stem cell sub-population

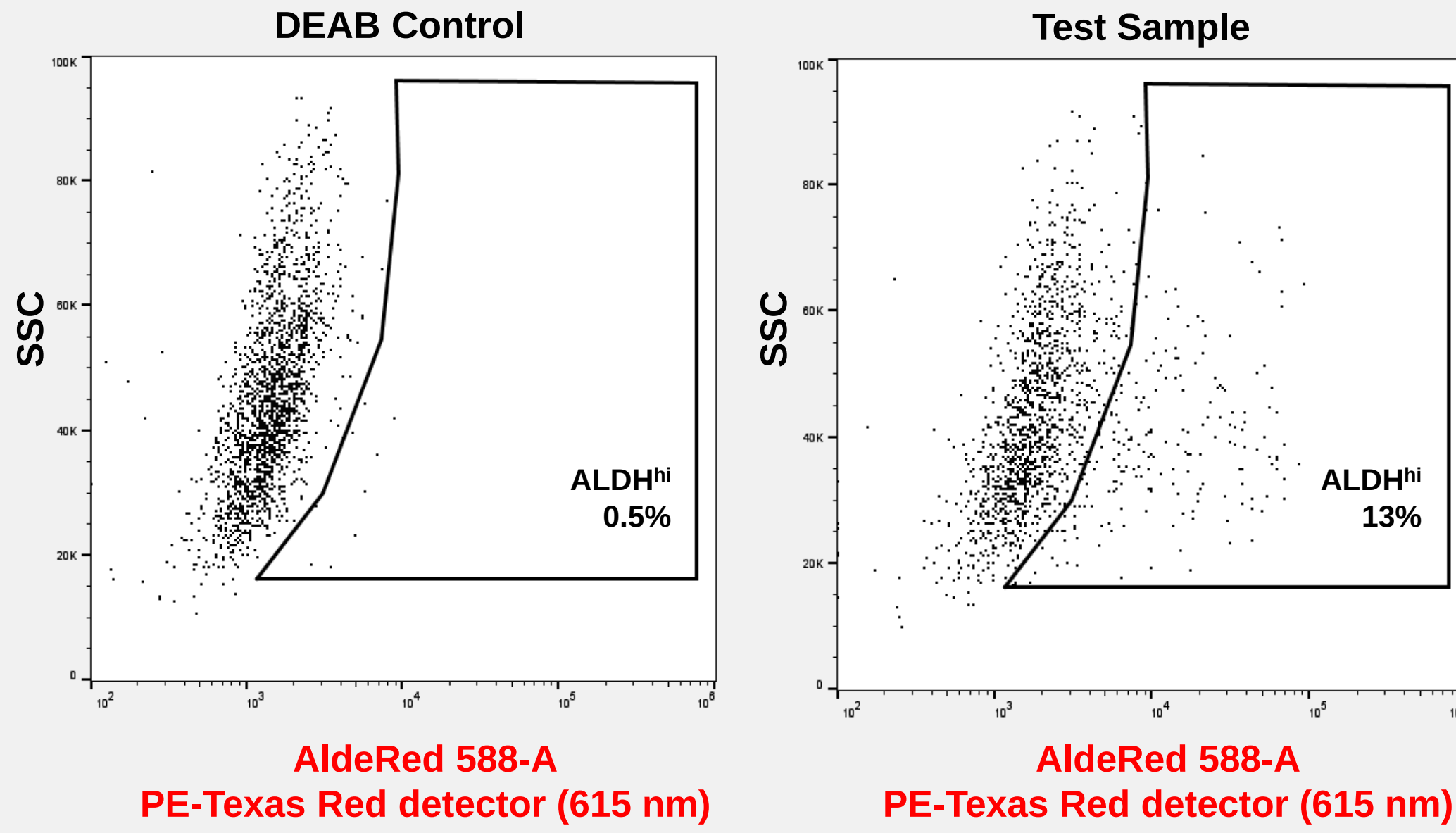


Figure 5. ALDH detection in UM-SCC-47 cancer cell line by AldeRed 588-A.

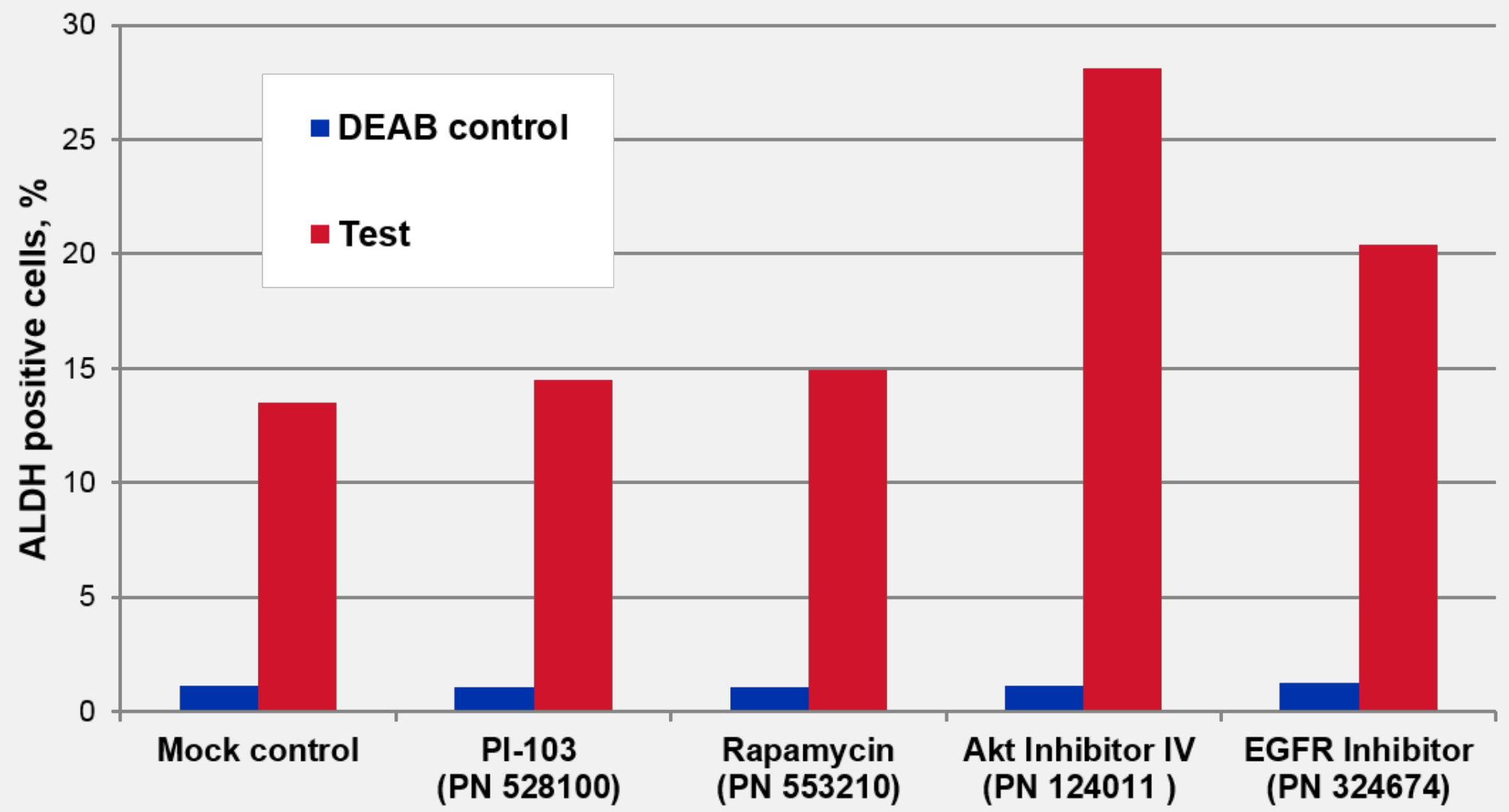


Figure 6. Effect of signaling pathway inhibitors on ALDH^{High} population. UM-SCC-47 cells were treated for 24 hrs with 1 uM of each inhibitor and ALDH activity was measured by AldeRed 588-A