Upload image

May 15, 2020

Isolation and identification of cancer stem cells by ALDH activity assay

Kwangok P Nickel[1], Austin M. Maas[1], Andrea Pennati[1], Randall Kimple[2]

[1]University of Wisconsin - Madison, [2]University of Wisconsin - Madison, UW Carbone Cancer Center

1 Works for me dx.doi.org/10.17504/protocols.io.bfyrjpv6

Randall Kimple

ABSTRACT

Cancer stem cells (CSCs) are a small subpopulation of tumor cells that are thought to be responsible for recurrence and metastasis of cancer due to their ability for self-renewal and differentiation into multiple cancer cell types.

Multiple methods have been used to identify CSCs using flow cytometry. Cell surface markers such as CD44 and CD133 can be used to identify the CSC population in tumor cells. Also, they can be isolated from a heterogeneous population using an assay that tests for the aldehyde dehydrogenase (ALDH) activity within the cell.

ALDH activity is measured by flow cytometer and expressed by percentage. A higher percentage indicates more CSCs are present in the sample. Aldehyde hydrogenases (ALDHs) are a group of enzymes that catalyze the oxidation of aldehydes to carboxylic acids. They are involved in detoxification of aldehydes produced by metabolic processes or cytotoxic drugs. An increase in ALDH activity may indicate cells are more resistant to chemotherapy. In addition, high ALDH activity has been shown in CSCs of different cancer types and is now considered to be a standard marker of CSCs. The Aldefluor assay has been used to identify and isolate cells with high ALDH activities. This assay is based on the principle that ALDH can convert the ALDH-substrate, Bodipy-aminoacetaldehyde (BAAA) into Bodipy-aminoacetate (BAA) which is retained inside the cells. BAAA is uncharged and can diffuse freely into intact viable cells, however BAA can not cross the membrane due to its net negative charge, which makes remain in the cells and the assay buffer prevents efflux of the BAA from the cells. Therefore, the amount of BAA fluorescence in cells is proportional to ALDH activity and can be measured using a flow cytometer. A specific inhibitor of ALDH, diethylaminobenzaldehyde (DEAB), is used for background fluorescence control.

GUIDELINES

Cells of interest (freshly collected primary tumor isolated from patient or animal or cell line of interest) prepared as a single cell suspension

MATERIALS

NAME	CATALOG #	VENDOR
ALDEFLOUR assay kit	01700	
Propidium Iodide - 1.0 mg/mL Solution in Water	P3566	Invitrogen - Thermo Fisher

MATERIALS TEXT

1.5 ml microfuge tubes, 5 ml flow tubes, Flow cytometer, Centrifuge with refrigeration or centrifuge in cold room.

1 Prepare activated ALDEFLUOR assay reagent, Bodipy-aminoacetaldehyde (BAAA), according to the manufacturer's directions **ALDEFLUOR assay kit manual.pdf**. Make **50 µl** aliquots and store them at **-20 °C** .

Note: In this example, a head and neck cancer cell line, UM-SCC47 **SCC071CA.pdf**, is analyzed. The experimental approach can be used to study cells isolated from primary human cancers or with established cancer cell lines. Also, Attune NxT (Thermo Fisher Scientific) is used as a choice of flow cytometer.

2 Trypsinize UM-SCC47 cells and count cells using a hemocytometer or automated cell counter. Cell numbers should be at least 3x 10^6 to prepare the following 6 tubes.

Citation: Kwangok P Nickel, Austin M. Maas, Andrea Pennati, Randall Kimple (05/15/2020). Isolation and identification of cancer stem cells byÃÂ ALDH activity assay . https://dx.doi.org/10.17504/protocols.io.bfyrjpv6

1. Unstained
2. PI only
3. Test (ALDH)
4. Control (DEAB)
5. Test (ALDH) without PI: FITC only
6. Control (DEAB) without PI: FITC only

3 Centrifuge cells at **250 x g** for 5 min to pellet, remove the supernatant and resuspend in ALDEFLUOR assay buffer to the final concentration of 1 x10^6 cell/ml in the both "Test" tubes. Prepare each Unstained and PI tube with 0.5 X 10^6 cells with 0.5 ml ALDEFLUOR assay buffer.

4 Add **5 µl** of diethylaminobenzaldehyde (DEAB), reagent provided as part of the ALDEFLUOR assay kit to both "Control" tubes. Close the tube immediately to prevent the evaporation of the DEAB solution (95% ethanol).

5 Add **5 µl** of activated ALDEFLUOR reagent to both "Test" tubes and mix by gentle vortexing or inversion.

6 Immediately transfer **0.5 ml** of mixture from each "Test" tube to each "Control" tube.

7 Incubate both "Test" and "Control" tubes for 45 min at **37 °C** in the dark. Do not exceed 60 min (incubation time should be optimized for cell type).

8 Following incubation, gently pellet cells by centrifugation at **250 x g** for 5 min.

9 Remove supernatant and resuspend cells in **0.5 ml** of ALDEFLUOR assay buffer. Transfer to 5 ml flow tubes and store the cells on ice or at **4 °C** in the dark.

10 Add **1 µl** of 1 mg/ml PI to PI, "Test "and "Control" tubes prior to flow analysis. Do not add PI to "Test" and "Control" FITC only tubes.

11 Perform flow cytometry.

11.1 Launch the Attune NxT software.

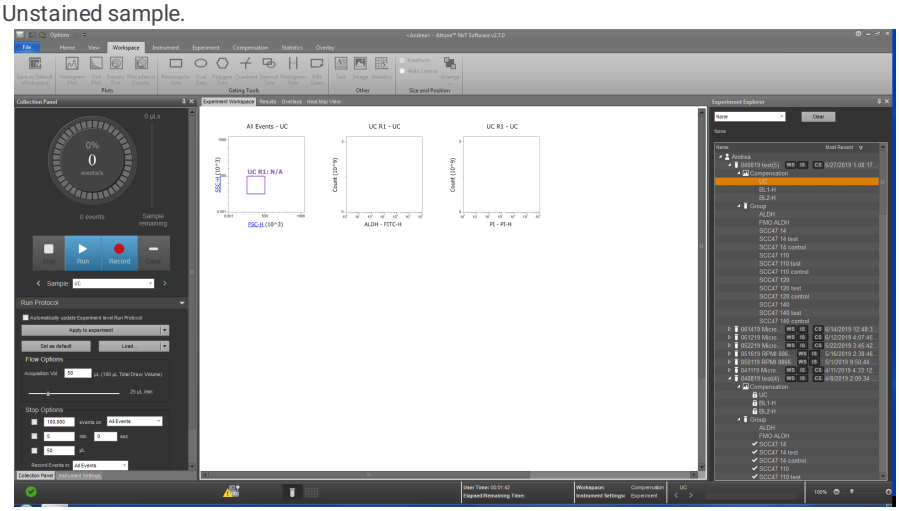
11.2 Create an Experiment.

1. On the main menu, click New Experiment.
2. Select Experiment type and enter Experiment name.

Citation: Kwangok P Nickel, Austin M. Maas, Andrea Pennati, Randall Kimple (05/15/2020). Isolation and identification of cancer stem cells byÃÂ ALDH activity assay . https://dx.doi.org/10.17504/protocols.io.bfyrjpv6

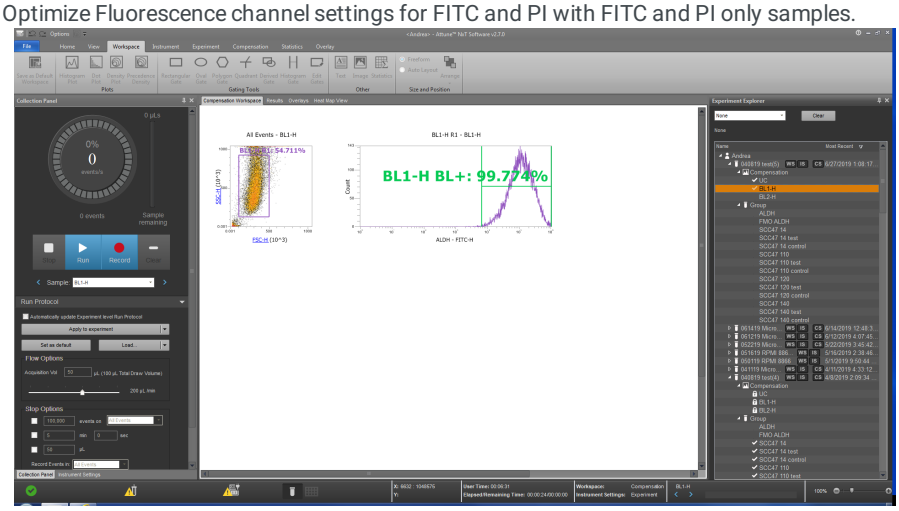
11.3 Compensation

1. Compensation setup is needed due to the spillover of FITC into PI caused by overlapping spectrum of FITC (ALDH positive cells will be detected in this channel) to PI.
2. Click Compensation Setup and select the source of compensation controls (BL1 for FTIC and BL2 for PI), the measurement parameter, background fluorescence mode and compensation parameters.
3. Optimize FSC (Forward Scatter) and SSC (Side Scatter) Voltage and Threshold settings for Unstained sample.



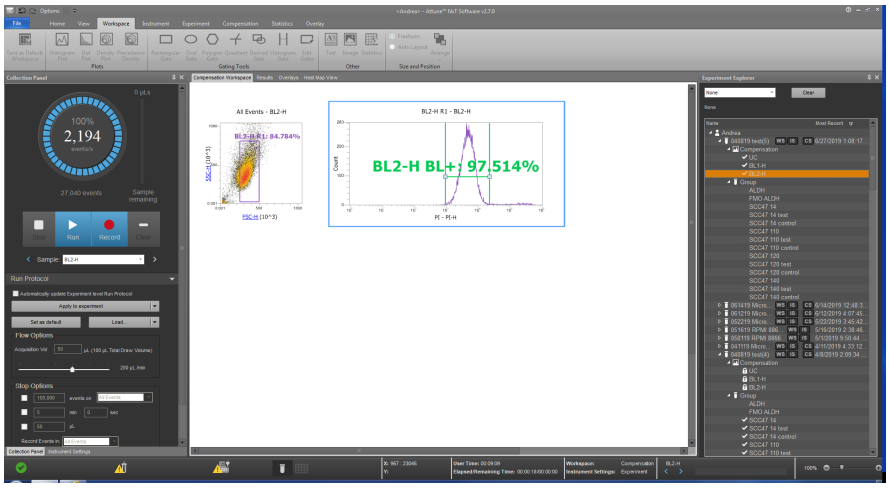
Compensation for Unstained sample

4. Optimize Fluorescence channel settings for FITC and PI with FITC and PI only samples.



Compensation for FITC

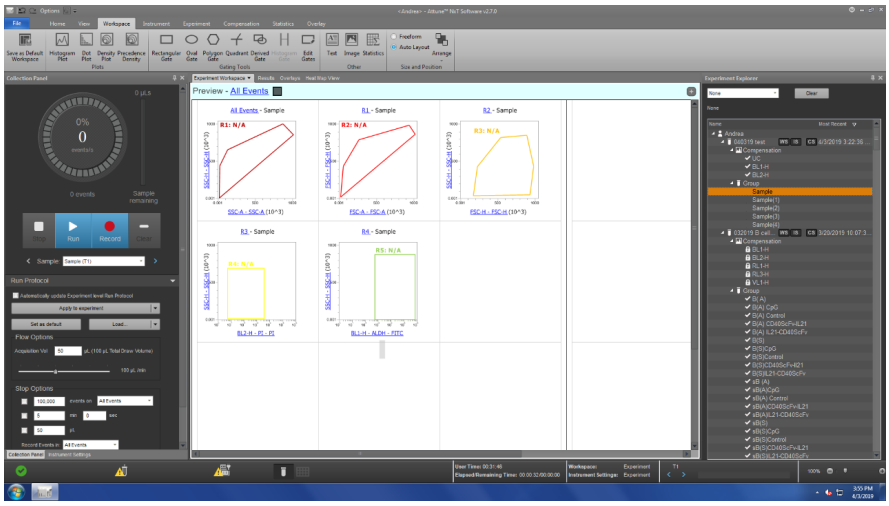
Citation: Kwangok P Nickel, Austin M. Maas, Andrea Pennati, Randall Kimple (05/15/2020). Isolation and identification of cancer stem cells byÃÂ ALDH activity assay . https://dx.doi.org/10.17504/protocols.io.bfyrjpv6



Compensation for PI

5. Record compensation.

12 Run sample.

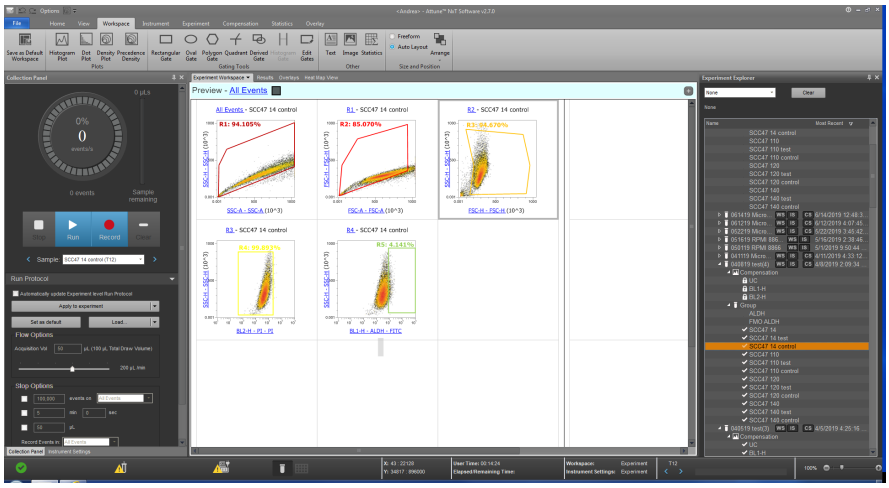


1. Set up for 5 dot plots.

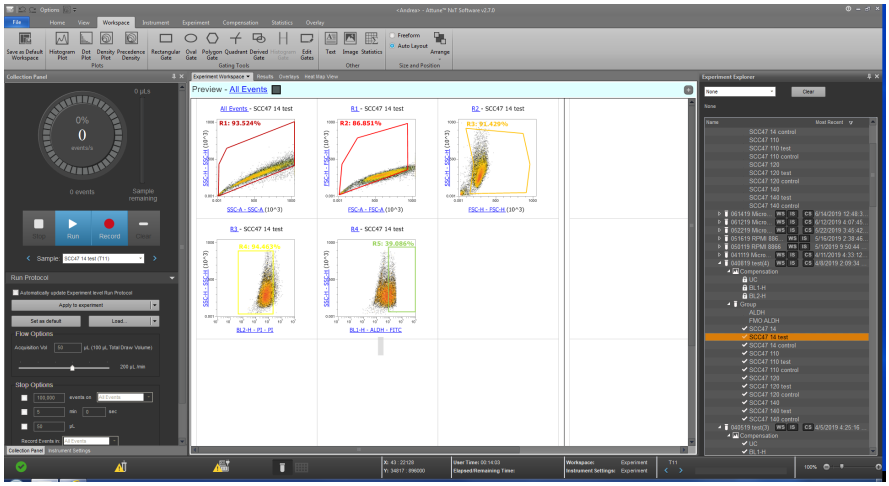
- dot plot 1: SSC-H (Height) vs. SSC-A (Area), to eliminate doublets
- dot plot 2: FSC-H vs. FSC-A, to eliminate doublets (second discrimination)
- dot plot 3: SSC-H vs. FSC-H, to select single cells
- dot plot 4: SSC-H vs. PI-H, to detect only live single cells
- dot plot 5: SSC-H vs. ALDH-H, to select ALDH+ cells

2. Run “Control (DEAB)” sample to set the gate of the ALDH positive population using the rightmost edge of the stained the “Control” DEAB population. This permits to identify the negative cells with no ALDH activity (ALDH-) and the positive cells with ALDH activity (ALDH+).Place the corresponding ALDH “Test” tube into the flow cytometer. Collect 100,000 events in live single cells gate.

Citation: Kwangok P Nickel, Austin M. Maas, Andrea Pennati, Randall Kimple (05/15/2020). Isolation and identification of cancer stem cells byÃÂ ALDH activity assay . https://dx.doi.org/10.17504/protocols.io.bfyrjpv6

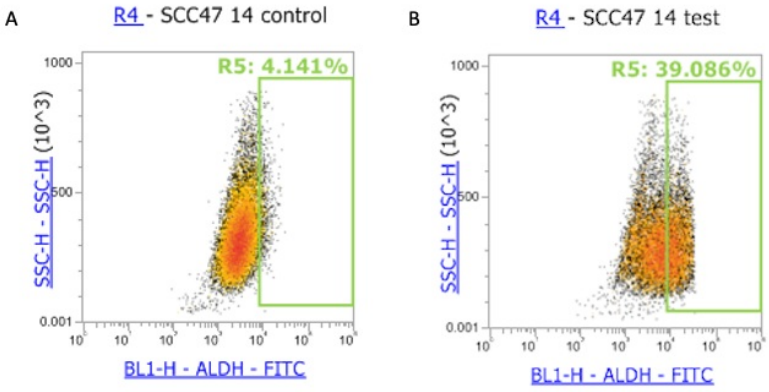


UM-SCC47 control (DEAB)



UM-SCC47 Test (ALDH)

13 In this figure, A: the Gate is set on the DEAB treated sample in order to include a small number of cells as ALDH positive (4.14%). B: a representative plot for UM-SCC47 cells is shown and demonstrates 39.09% of the population is ALDH-positive.



Percentage of ALDH+ CSCs in UM-SCC47 cells

Citation: Kwangok P Nickel, Austin M. Maas, Andrea Pennati, Randall Kimple (05/15/2020). Isolation and identification of cancer stem cells byÃÂ ALDH activity assay . https://dx.doi.org/10.17504/protocols.io.bfyrjpv6

We typically perform a minimum of 3 biologic replicates (3 independent experiments) and average the present positive cells from the 3 replicates. When this approach is taken, the mean and standard deviation should be reported.

Citation: Kwangok P Nickel, Austin M. Maas, Andrea Pennati, Randall Kimple (05/15/2020). Isolation and identification of cancer stem cells byÃÂ ALDH activity assay . https://dx.doi.org/10.17504/protocols.io.bfyrjpv6