AB308318 – Aldehyde Dehydrogenase 3A1 Inhibitor Screening Kit (F)

Screening and characterizing potential ALDH3A1 inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: abcam website

Storage and Stability

Upon arrival, store kit at -20 °C, protected from light. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

Materials Supplied

Item	Quantity	Storage Condition
ALDH3A1 Assay Buffer	25 ml	-
Recombinant ALDH3A1	1 vial	-20 °C
ALDH3A1 Substrate	50 µl	-20 °C
ALDH3A1 Substrate Mix	1 vial	-20 °C
PicoProbe™ (Avoid light)	0.4 ml	-20 °C
ALDH3A1 Inhibitor (Avoid light)	1 vial	-20 °C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 1N NaOH
- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (plate reader)
- Dounce Tissue Homogenizer
- 10 kD Spin Column

Reagent Preparation

ALDH3A1 Assay Buffer and PicoProbe™ (in DMSO): Warm to room temperature (RT) before use.

Recombinant ALDH3A1: Provided as liquid. Keep on ice until it thaws completely. Divide into aliquots and store at -20 °C. Avoid repeated freeze/thaw cycles.

ALDH3A1 Substrate: Warm to RT before use. Store at -20 °C.

ALDH3A1 Substrate Mix: Reconstitute the vial in 220 µl ALDH3A1 Assay Buffer. Pipette up and down to dissolve the contents completely. Keep on ice and store at -20 °C.

ALDH3A1 Inhibitor: Reconstitute the vial in 20 µl DMSO. Divide into aliquots and store at -20 °C, protected from light. Stock solutions are stable for up to 3 months at -20 °C. Warm to RT before use.

ALDH3A1 Inhibitor Screening Protocol

Recombinant ALDH3A1:

Dilute Recombinant ALDH3A1 at 1:250 dilutions with ALDH3A1 Assay Buffer (i.e., for 20 reactions, dilute 2 µl ALDH3A1 into 498 µl ALDH3A1 Assay Buffer). Mix thoroughly and keep on ice. Add 25 µl of diluted ALDH3A1 into desired wells of a 96-well white plate labeled as Sample (S), Solvent Control (SC), Inhibitor Control (IC) and Enzyme Control (EC) and Background Control (BC) respectively.

Screening Test Inhibitor(s):

Dissolve the Test Inhibitor(s) in an appropriate solvent to make 100X stock solution. Dilute the stock Test Inhibitor to 4X using ALDH3A1 Assay Buffer. Add 25 µl of diluted Test Inhibitor(s) into Sample (S) well(s). Add 25 µl of 4X Solvent (1X final well solvent concentration) into the Solvent Control (SC) well.

Δ Note:

1. *Solvents used to solubilize the Test Inhibitor(s) might affect the enzymatic activity. To determine the effect of the solvent on ALDH3A1 activity, we recommend preparing a Solvent Control (SC) well with the same final concentration of solvent used to dissolve the Test Inhibitor(s).*
2. *IC_{50} estimation (Optional): Prepare several dilutions of the Test Inhibitor(s) in ALDH3A1 Assay Buffer while maintaining consistent final Solvent Concentration in all wells. Add 25 µl of each dilution into the designated wells.*

Enzyme, Background and Inhibitor Controls:

Add 25 µl of ALDH3A1 Assay Buffer to both EC and BC wells. Add 2 µl of ALDH3A1 Inhibitor to the IC well and adjust the volume to 25 µl using ALDH3A1 Assay Buffer. At this stage, all wells including S, SC, IC, EC and BC contain 50 µl reaction volumes. Incubate the plate at RT for 10 min, protected from light.

Reaction Mix Preparation:

Make sufficient Reaction Mix volume for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

	Reaction Mix	Background Mix
ALDH3A1 Assay Buffer	45.5 µl	46 µl
PicoProbe™	2 µl	2 µl
ALDH3A1 Substrate Mix	2 µl	2 µl
ALDH3A1 Substrate	0.5 µl	---

Mix well. Add 50 µl Reaction Mix to S, SC, IC and EC wells and 50 µl Background Mix to BC well respectively. The total reaction volume is 100 µl for all wells.

Measurement:

Measure the fluorescence in kinetic mode at Ex/Em = 535/587 nm at 3 min intervals for 45 min at RT.

Calculation:

Subtract the RFU of BC well from S, EC, SC and IC wells. Obtain ΔRFU for S, EC, SC and IC by subtracting RFU at time t_1 from RFU at time t_2 such that t_2 and t_1 is within the linear range of the assay. If [SC] ΔRFU is significantly different when compared to [EC] ΔRFU, use [SC] ΔRFU in the formula below instead of [EC] ΔRFU to determine the effect of Test Inhibitor. Calculate the percentage inhibition as shown below:

% Relative Inhibition = (1- $(\Delta RFU(S))/(\Delta RFU(EC))$ **) X 100**

Technical Support

For all technical or commercial enquiries please go to:
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