

Aldehyde Dehydrogenase 3A1 Inhibitor Screening Kit (F)

10/20

(Catalog # K2060-100; 100 assays; Store at -20 °C)

I. Introduction:

Aldehyde Dehydrogenases (ALDHs; EC 1.2.1.3) are oxidizing enzymes that play an important, anti-oxidative role by oxidizing aldehydes generated from the metabolism of amino acids, fatty acids, food, xenobiotic drugs etc. to carboxylic acids. Aldehyde Dehydrogenase 3A1 (ALDH3A1), a member of the ALDH superfamily is highly expressed in various cancers such as hepatoma, lung adenocarcinoma, myeloma, breast cancer etc. It has been shown to play an important role in the survival, metastasis, and chemo-resistance of cancer cells. Thus, the selective inhibition of ALDH3A1 in cancer cells could restore chemosensitivity thereby allowing lower effective dosages and reducing unwanted side effects. **BioVision's ALDH3A1 Inhibitor Screening Kit** provides a quick and sensitive way for screening, studying and characterizing the potential inhibitors of ALDH3A1. In this assay, ALDH3A1 oxidizes a specific substrate and generates NADH as one of the intermediate. NADH then couples with the PicoProbe™ to form a stable fluorophore, which is measured at Ex/Em = 535/587 nm. ALDH3A1 Inhibitor Control is included in the kit to compare the efficacy of test inhibitors. The assay is high-throughput adaptable and can be performed within 30-60 min.

II. Application:

- Screening and characterizing potential ALDH3A1 inhibitors.

III. Kit Contents:

Components	K2060-100	Cap Code	Part Number
ALDH3A1 Assay Buffer	25 ml	WM	K2060-100-1
Recombinant ALDH3A1	1 vial	Green	K2060-100-2
ALDH3A1 Substrate	50 µl	Purple	K2060-100-3
ALDH3A1 Substrate Mix	1 vial	Red	K2060-100-4
PicoProbe™	0.4 ml	Blue	K2060-100-5
ALDH3A1 Inhibitor	1 vial	Amber	K2060-100-6

IV. User Supplied Reagents and Equipment:

- DMSO
- 96-well white plate with flat bottom (low/medium binding)
- Multi-well spectrophotometer (Fluorescent plate reader)

V. Storage Conditions and Reagent Preparation:

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.

- **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.

VI. ALDH3A1 Inhibitor Screening Protocol:

1. 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.

2. 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.

Notes:

a). 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).

b). **IC₅₀ 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.

3. 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.**

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

	<u>Reaction Mix</u>	<u>Background Mix</u>
ALDH3A1 Assay Buffer	45.5 μ l	46 μ l
PicoProbe™	2 μ l	2 μ l
ALDH3A1 Substrate Mix	2 μ l	2 μ l
ALDH3A1 Substrate	0.5 μ l	--

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

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

6. Calculation: Subtract the RFU of **BC** well from **S**, **EC**, **SC** and **IC** wells. Obtain Δ RFU 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**] Δ RFU is significantly different when compared to [**EC**] Δ RFU, use [**SC**] Δ RFU in the formula below instead of [**EC**] Δ RFU to determine the effect of Test Inhibitor. Calculate the percentage inhibition as shown below:

$$\% \text{ Relative Inhibition} = \left(1 - \frac{\Delta\text{RFU}(S)}{\Delta\text{RFU}(EC)}\right) \times 100$$

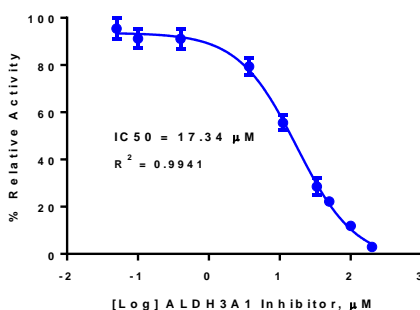


Figure: Inhibition of ALDH3A1 activity using ALDH3A1 inhibitor. IC₅₀ was calculated to be 17.34 \pm 0.54 μ M. Assay was performed following the kit protocol.

VII. Related Products:

Aldehyde Dehydrogenase 2 Inhibitor Screening Kit (Fluorometric) (Cat. # K2011-100)
 Aldehyde Dehydrogenase Activity Colorimetric Assay Kit (Cat. # K731-100)
 PicoProbe™ Aldehyde Dehydrogenase Activity Fluorometric Assay Kit (Cat. # K741-100)
 Aldehyde Dehydrogenase, Mitochondrial (ALDH2) (Human) ELISA Kit (Cat. # E4587-100)
 Human Recombinant ALDH2 (Cat. # 6332-100)
 Human Recombinant ALDH3A1 (Cat. # 6333-50)
 Anti-ALDH1A1 Antibody (ALDH1A1/1381) (Cat. # A1424-100)
 ALDH2 Antibody (Clone # 138CT22.3.8) (Cat. # 6746-100)
 ALDH2 Antibody (Cat. # 6747-100)
 ALDH2 Antibody (Center) (Cat. # 6748-100)
 ALDH2 Antibody (NT) (Cat. # 6749-100)
 ALDH5A1 Antibody (Cat. # 6750-100)
 ALDH5A1 Antibody (CT) (Cat. # 6751-100)
 ALDH5A1 Antibody (NT) (Cat. # 6752-100)
 Disulfiram (Cat. # 2308-50, 250)
 A37 (Cat. # B1577-5, 25)

FOR RESEARCH USE ONLY! Not to be used on humans.