

Aldehyde Dehydrogenase 2 Inhibitor Screening Kit (Fluorometric)

07/20

(Catalog # K2011-100; 100 assays; Store at -20°C)

I. Introduction:

Aldehyde Dehydrogenase 2 (ALDH2), mitochondrial is a member of the ALDH superfamily that participates in multiple metabolic pathways including the oxidation of toxic biogenic and environmental aldehydes. ALDH2 is a key enzyme located in the mitochondrial matrix. It plays an important role in ethanol metabolism by converting acetaldehyde, a carcinogenic metabolite of ethanol to acetate. Imbalances of ALDH2 have been linked to both alcoholism and alcohol sensitivity in people. Reduced ALDH2 activity has been strongly correlated with reduced alcohol consumption and reduced incidences of alcoholism, which led to the development of selective ALDH2 inhibitors as antidipsotropic or alcohol aversive agents for treating alcoholism. Disulfiram, an alcohol-aversive drug inhibiting ALDH2 has been approved by the FDA and is used clinically for over 40 years for treating alcohol dependency. However, the drug is a non-specific alkylating agent leading to many adverse side effects and drug-drug interactions. Thus, novel and specific inhibitors of ALDH2 free of the negative side effects could be used in aversion therapy. **BioVision's Aldehyde Dehydrogenase 2 Inhibitor Screening Kit** provides a quick and sensitive way of screening, studying and characterizing potential inhibitors of ALDH2. In this assay, acetaldehyde is oxidized by ALDH2 to form NADH, which then couples with PicoProbe™ to generate a fluorescence signal measured at Ex/Em = 535/587 nm. The percentage inhibition can be determined by comparing the activity of ALDH2 treated with test inhibitor(s) vs untreated control.

II. Application:

- Screening or characterizing ALDH2 inhibitors.

III. Kit Contents:

Components	K2011-100	Cap Code	Part Number
ALDH2 Assay Buffer	25 ml	WM	K2011-100-1
Recombinant ALDH2	50 µl	Green	K2011-100-2
ALDH2 Substrate	0.5 ml	Purple	K2011-100-3
ALDH2 Substrate Mix	1 vial	Red	K2011-100-4
PicoProbe™	0.4 ml	Blue	K2011-100-5
Inhibitor (Disulfiram, 2 mM)	50 µl	Yellow	K2011-100-6

IV. User Supplied Reagents and Equipment:

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

V. Storage Conditions and Reagent Preparation:

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

- **ALDH2 Assay Buffer and PicoProbe™:** Warm to room temperature (RT) before use.
- **Recombinant ALDH2:** Keep on ice until it dissolves. Divide into aliquots and store at -20°C. Avoid repeated freeze/thaw cycles.
- **ALDH2 Substrate:** Keep on ice until it dissolves completely. Store at -20°C.
- **ALDH2 Substrate Mix (Lyophilized):** Reconstitute the vial in 220 µl ALDH2 Assay Buffer. Pipette up and down to dissolve completely. Keep on ice and store at -20°C.
- **Inhibitor (Disulfiram, 2 mM):** Ready to use. Warm to RT before use. Divide into aliquots and store at -20°C.

VI. ALDH2 Inhibitor Screening Protocol:

1. Recombinant ALDH2: Dilute the recombinant ALDH2 at 1:10 with ALDH2 Assay Buffer. Mix thoroughly and keep on ice. Add 5 µl of diluted recombinant ALDH2 into the desired wells of a 96-well white plate labeled as Sample (**S**), Solvent Control (**SC**), Inhibitor Control (**IC**), Enzyme Control (**EC**) and Background Control (**BC**) respectively. Adjust the volume of all wells to 25 µl with ALDH2 Assay Buffer.

2. Screening Test Inhibitor(s): Dissolve Test Inhibitor(s) in an appropriate solvent to make 100X stock solution. Dilute the stock Test Inhibitor to 4X using ALDH2 Assay Buffer. Add 25 µl of diluted Test Inhibitor(s) into the Sample (**S**) well(s). Add 25 µl of 4X Solvent (1X final well solvent concentration) into the **SC** well.

Note: Solvents used to solubilize the Test Inhibitor(s) might affect the enzymatic activity. Prepare a Solvent Control well with the same final concentration of Solvent used to dissolve the Test Inhibitor(s).

3. Enzyme, Background and Inhibitor Control Preparation: Add 25 µl of ALDH2 Assay Buffer to the **EC** and **BC** wells. Add 2 µl of Inhibitor (**Disulfiram**) to the Inhibitor Control (**IC**) well and bring up the volume to 25 µl/well using 23 µl of ALDH2 Assay Buffer. **IC₅₀ estimation (Optional):** Prepare several dilutions of the Test Inhibitor(s) in ALDH2 Assay Buffer maintaining consistent final Solvent Concentration in all wells. Add 25 µl of each dilution into the designated wells. At this stage, all wells including S, SC, IC, EC and BC contain 50 µl. **Incubate for 30 min at RT.**

4. Reaction Mix Preparation: Make enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	<u>Reaction Mix</u>	<u>Background Control Mix</u>
ALDH2 Assay Buffer	45.5 µl	46 µl
PicoProbe™	2 µl	2 µl
ALDH2 Substrate Mix	2 µl	2 µl
ALDH2 Substrate	0.5 µl	----

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

5. Measurement: Measure fluorescence in a kinetic mode at Ex/Em = 535/587 nm at 3 min intervals for 30-60 min at RT.

6. Calculation: Subtract the RFU of the BC well from all Test Samples (**S**), Enzyme Control (**EC**), Solvent Control (**SC**) and Inhibitor Control (**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 a linear range of the assay. If Δ RFU of Solvent Control [**SC**] is significantly different from Δ RFU of Enzyme Control [**EC**], use its values instead of **EC** in the calculations shown below.

Calculate ALDH2 Inhibition for Test Inhibitor(s) as below:

$$\% \text{ Relative Inhibition} = \frac{\Delta\text{RFU}[\text{EC}] - \Delta\text{RFU}[\text{S}]}{\Delta\text{RFU}(\text{EC})} \times 100$$

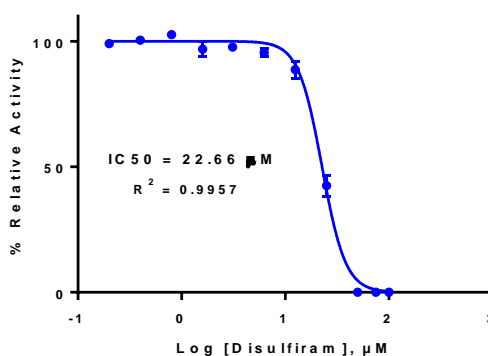


Figure: Inhibition of ALDH2 activity by Disulfiram, an alcohol dehydrogenase inhibitor (BioVision Cat# 2308). IC₅₀ of Disulfiram was calculated to be 22.66 ± 1.36 μM. Assay was performed following the kit protocol.

VII. Related Products:

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)

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