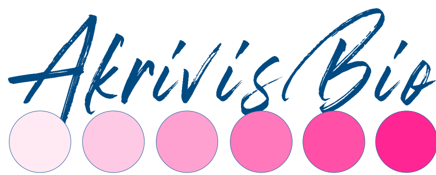


MA-0169

Aldehyde Dehydrogenase Activity Assay

(100 wells, Colorimetric, OD 450 nm, Store Kit at -20°C)

Background:

Aldehyde Dehydrogenase is primarily known for its role in alcohol detoxification with the conversion of acetaldehyde to acetate. It has a number of lesser-known functions beyond that as a key ingredient in detoxification of endogenous products during lipid metabolism and oxidative stress; as an important influence in neuroprotection (removal of aldehydes formed during dopamine catabolism) and in retinal health; protection off the heart against ischemic injury. Dysregulation and elevated levels are found in a variety of cancers where it appears to contri bute to resistance to chemotherapy and in promoting tumor progression. This wide range of activities contributed by a number of different isotypes makes the ability to monitor ALDH levels important. AkrivisBio's Aldehyde Dehydrogenase Activity Assay is a very simple, sensitive way to monitor ALDH activity in a variety of sample types with a sensitivity below 50 µUnits.

Assay Components:

Assay Buffer	25 ml	WM	MA-0169A
Acetaldehyde	0.5 ml	Purple	MA-0169B
WST-8 Reagent	Lyoph	Red	MA-0169C
ALDH Positive Control	Lyoph	Green	MA-1069D
NADH Standard	Lyoph	Yellow	MA-0169E

User supplied Materials:

Glycerol

Storage and Handling:

Store unopened kit at -20ºC. Allow all components to come to room temperature prior to use. Centrifuge all vials briefly, prior to opening ro recover materials that may have become trapped around the cap.
Assay Buffer: Ready to use as supplied. Store at -20°C
Acetaldehyde: Supplied as a 100 mM solution. Store at -20°C.
WST-8 Reagent Mix: Reconstitute with 220 µl DI H_2O, then let sit undisturbed for 2-3 minutes. Vortex very briefly to complete dissolution. Store at –20ºC.
ALDH Positive Control: Add 20 µl of glycerol to 80 µl of Assay Buffer and mix well. Reconstitute ALDH with the Assay Buffer/20% glycerol mixture, aliquot into convenient portions and store at -20°C to avoid repeated freeze/thaw cycles.
NADH Standard: Supplied as 200 nmoles lyophilized material**.** Reconstitute with 500 µl DI H_2O giving an 0.4 mM solution. Store at -20°C.

Assay Protocol:

1. **NADH Standard Curve:** Transfer 0 – 5 – 10 – 15 – 20 - 25 µl into a series of wells in a 96 well plate, giving 0, 2, 4, 6, 8, 10 nmoles NADH, respectively. Adjust all well volumes to 50 µl with Assay Buffer.
2. **Sample Preparation:** Transfer 2 – 50 µl of liquid samples directly to the plate. Homogenize tissue (10 mg) or cells (1 x 10^6) in 200 µl ice cold Assay Buffer on ice. Centrifuge the homogenate at 20,000 X g to pellet cell debris and transfer the supernatant to a fresh tube. Transfer 2 – 50 µl of the supernatant to the plate and adjust all sample wells to 50 µl with Assay Buffer.
3. **Positive Control (Optional):** If you wish to have a positive control well, transfer 10 µl of the reconstituted ALDH to a well(s) and adjust the volume to 50 µl with Assay Buffer.

Note: All sample readings should be within the range of the standard curve. If any sample exceeds that range within 5 minutes of starting the reaction, dilute the sample appropriately and rerun. NADH in samples gives an elevated but constant background. As long as it is not above ~ 0.3 OD, it will not interfere with the enzyme activity assay.

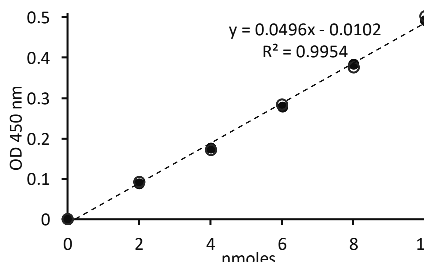
4. **Initiate Reaction**: Each Sample and Positive Control well will require 50 µl of Reaction Mix containing acetaldehyde. Standards will require 50 µl of Reaction Mix without the acetaldehyde. Prepare sufficient materials for the number of wells to be analyzed containing:

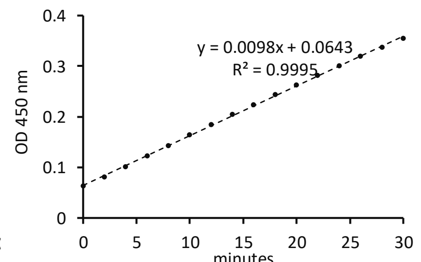
	Sample & Positive Control Wells	Standards
Assay Buffer	43 µl	48 µl
WST-8 Reagent	2 µl	2 µl
Acetaldehyde	5 µl	----

Add 50 µl of the Reaction Mix to each well containing the Standard, test samples and background controls, mix well.

5. **Measurement:** Place the plate into a plate reader and monitor the reaction progress at 450 nm at room temperature for 15 – 120 minutes. The Standards will develop color rapidly and then show a constant absorbance for the duration of the experiment. The samples and positive control well should show a constantly increasing absorbance as the reaction proceeds. Ideally the reaction will proceed i n a smooth linear manner with little to no curvature.
6. **Typical Results:**

nmoles Standard	Standard Raw Values (OD)		Background Corrected Values (OD)	
0	0.0650	0.0631	0	0
2	0.1535	0.1560	0.0885	0.1069
4	0.2416	0.2353	0.1766	0.1722
6	0.3441	0.3467	0.2791	0.2836
8	0.4505	0.4384	0.3855	0.3753
10	0.5577	0.5656	0.4927	0.5025





7. **Calculation:** Subtract the 0 Standard reading from all Standard readings and plot the Standard Curve. Determine the slope of the Standard Curve (OD/nmole). Identify a linear region for the sample reactions. Determine the slope of the sample reactions (OD/minute). Divide the slope of each sample reaction by the slope of the Standard Curve to convert the sample rates to nmoles/minute (mUnits). This is the activity in each sample well. To convert that back to activity in the original sample:

Enzyme rate in well (Step **7**) X (total volume of extract (from step **2**) / volume added to well) = Total Activity from Sample.

Total Activity from Sample / mg of raw sample (µl of fluid sample before any dilution) = activity as (mU)/mg or µl of sample

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