MA-0162

Alcohol Dehydrogenase Activity Assay

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

Introduction:

Alcohol dehydrogenase plays a small metabolic role in humans but is a much more important contributor to the health and survivability of microorganisms such as yeast and bacteria. In humans ADH resides primarily in the liver where it detoxifies ethanol. It's role in microorganisms is more interesting as it is involved in anaerobic energy production, adaptability to toxic environments bioremediation. ADH generates NADH in the process of oxidizing alcohol, an important intermediate in energy production. AkrivisBio's Alcohol Dehydrogenase Assay provides a simple, sensitive means of quantifying ADH activity in a variety of sample types with a lower limit of detection in the low microunit range.

Assay Principle:

- ADH oxidizes ethanol with the conversion of NAD to NADH
- NADH is used to reduce an almost colorless tetrazolium into a deeply colored formazan.

Kit Components:

Assay Buffer	25 ml	WM	MA-0162A
Ethanol	1 ml	Blue	MA-0162B
WST-8 Reagent	Lyoph	Red	MA-0162C
ADH Positive Control	Lyoph	Green	MA-0162D
NADH Standard	Lyoph	Yellow	MA-0162E

Storage and Handling Considerations:

Store the kit at -20ºC, protect from light. Allow ADH Assay Buffer to warm to room temperature before use. Briefly centrifuge vials prior to opening. Read the entire protocol before performing the assay.
Store the unopened assay at -20°C. Bring all components to room temperature before using. Centrifuge all vials briefly before opening.
Assay Buffer: Ready to use as supplied. Store at 4°C.
Ethanol: Keep tightly closed to avoid absorption of water, changing the ethanol concentration.
WST-8 Reagent: Reconstitute with 900 µl of DI water. Replace the lid and let it sit undisturbed for a few minutes to dissolve. Vortex gently for a few seconds and it is ready for use. Store at 4°C.
ADH Positive Control: Reconstitute with 220 µl Assay Buffer. Keep on ice while in use. If it is going to be used multiple times over a period of several days to a few weeks, aliquot to convenient portions and store at -20ºC.
NADH: Reconstitute with 50 µl of DI water. Keep on ice while in use. Store at -20ºC

Assay Protocol:

1. Prewarm a plate reader to 37°C, the temperature at which the assay will be run. Warm the Assay Buffer to 37°.

2. Standard Curve: Add 10 µl of the NADH stock solution to 90 µl of DI water, giving an 0.4 mM solution. Transfer 0 – 5 – 10 – 15 – 20 – 25 µl of the NADH to a 96-well plate, giving 0, 2, 4, 6, 8, 10 nmoles respectively. Adjust all wells 50 µl with Assay Buffer.

3. Samples: Homogenize tissue (10 mg) or cells (1 x 10^6) in 100 µl of ice-cold Assay Buffer. Centrifuge at 16,000 x g, 10 min to remove cell and other debris. Transfer the clear supernatant to a fresh tube. Load up to 50 µl of each sample into a 96-well plate. Fluid samples (serum, plasma, saliva, etc.) can be loaded directly to the 96-well plate. It is important that all sample readings are within the range of the standard curve. If any sample is outside of that range, dilute appropriately and rerun. Adjust all sample wells to 50 µl with Assay Buffer.

4. Positive Control: Add 1 - 5 µl of reconstituted Positive Control to well(s). Adjust the wells to 50 µl with Assay Buffer.

5. Background Control: Some samples may contain constituents which cause a significant background. In that event, rerun the sample in duplicate with one of the pair being used as a background control.

6. Initiate Reaction: Each Standard, Sample, Positive Control and Background Control well will require 100 µl of Reaction Mix**.** Prepare sufficient material for the total number of wells to be analyzed containing:

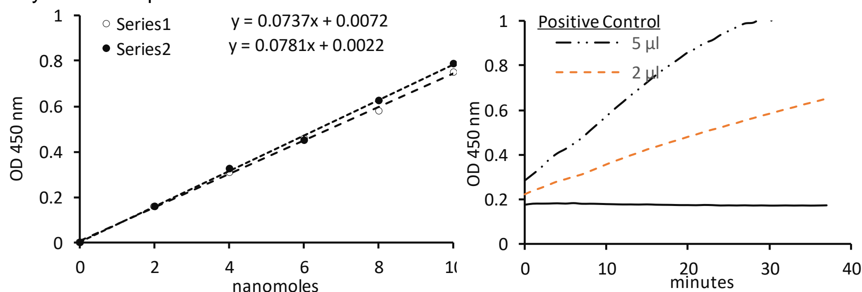
	Reaction Mix	Background Control
Assay Buffer	82 µl	92 µl
WST-8 Reagent	8 µl	8 µl
Ethanol	10 µl	------

Add 100 µl of the Reaction Mix to each well containing Standards, Samples or Positive Controls.

7. Measurement: Monitor the reaction in a plate reader, previously set to 37°C, at 450 nm in kinetic mode. Set the maximum time to 3-4 hours. The actual rection time will depend on the ADH activity in the samples. The lowest activities should achieve an increase of at least 0.1-0.2 OD above the absorbance at t_0.

8. Typical Results:

nmol Standard	Standard Raw Values		Background Corrected Values	
	OD			
0	0.1681	0.1620	0	0
2	0.326	0.3238	0.1579	0.1618
4	0.4773	0.4889	0.3092	0.3269
6	0.6275	0.6131	0.4594	0.4511
8	0.7462	0.7885	0.5781	0.6265
10	0.9176	0.9518	0.7495	0.7898



9. Calculation: Subtract the 0 Standard value from all other standards. Plot the Standard Curve. Determine the slope of the Standard Curve. If there are Background Control wells, determine the slopes of the background control wells and the sample wells. Subtract the slope of the background control well from the slope of its paired sample well. If there are no background control wells, just use the slope of the sample well. Divide the slopes of the sample wells (OD/min) by the slope of the Standard Curve to convert to nanomoles/minute in the wells. To convert back to activity in the original samples:
- Divide the rate in the well (nmoles/min) by the volume of sample applied to the well = nanomoles / min / µl of sample (mU / µl).
- Multiply the mU / µl of sample by the total volume of supernatant recovered in step 3 above = total mU in the original sample.
- Divide the total mU in the original sample by the mg tissue (or # of cells) used to prepare the sample = mU / mg tissue (/ # of cells, etc.)

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