MA-0131

Ethanol Assay

(100 wells, Colorimetric, OD 570 nm or Fluorometric, Ex/Em = 535/587 nm, Store at -20°C)

Background Information:

Ethanol plays a role in various metabolic pathways within the human body. When consumed, it is metabolized primarily in the liver via oxidative metabolism, involving alcohol dehydrogenase and acetaldehyde dehydrogenase, converting ethanol through toxic acetaldehyde, to acetate and eventually CO_2 and water. Ethanol metabolism affects energy balance and nutrient utilization. The metabolism of ethanol takes priority over other macronutrients, leading to storage of excess carbohydrates and fats. Chronic alcohol consumption disrupts metabolic pathways, impacting liver function, lipid metabolism, and glucose regulation. AkrivisBio's Ethanol Assay provides a simple, sensitive method for precise measurement of ethanol in a variety of samples. In the assay, alcohol oxidase oxidizes ethanol forming H_2O_2 which is used to form resorufin with intense color and fluorescence with sensitivity to 0.05 nmol.

Assay Principle:

1 – Ethanol is oxidized by alcohol oxidase forming acetaldehyde and hydrogen peroxide
2 – Peroxidase uses the peroxide to oxidize ADHP to resorufin with the generation of color and fluorescence.
Note: Alcohol in the air will adversely affect the performance of the assay. It is critical that the assay be performed in a completely alcohol free environment.

Assay Components:

Assay Buffer	25 ml	WM	MA-0131A
ADHP Solution	200 µl	Red	MA-0131B
Alcohol oxidase/Peroxidase	lyoph	Green	MA-0131C
Ethanol Standard (40 mM)	0.2 ml	Yellow	MA-0131D

Storage and Handling:

Store kit at –20°C prior to use. Warm Assay components to room temperature before use. Centrifuge all small vials for several seconds prior to opening.
Assay Buffer: Ready to use as supplied. Store at 4°C. Keep tightly closed when not in use.
ADHP Solution: Ready to use as supplied. Store at –20°C.
Alcohol oxidase/Peroxidase: Add 220 µl Assay Buffer to the enzyme mixture. Store at 4°C.
Ethanol Standard: Ready to use as supplied. Store at -20°C. Open only to withdraw a sample. Keep tightly closed when not in use.

Assay Protocol:

1. Standard Curve: a. Absorbance based assay: Transfer 10 µl of ethanol standard to 990 µl Assay Buffer and mix, giving an 0.4 mM working solution. Transfer 0–5–10–15–20–25µl to a series of wells in a 96 well plate. Adjust all well volumes to 50 µl with Assay Buffer, giving 0–2–4–6–8-10 nmol per well of the ethanol standard. **b. Fluorescence based assay:** Dilute the standard as in the absorbance based assay, then dilute further by transferring 10 µl into 190 µl of Assay buffer giving a 20 µM working solution. Transfer 0 – 5 – 10 – 15 – 20 – 25 µl to a series of wells in a 96 well plate. Adjust all well volumes to 50 µl with Assay Buffer, giving 0 – 100 – 200– 300 – 400 - 500 pmol per well of the ethanol standard.

2. Sample Preparation: All sample readings must fall within the range of the standard curve and should be prepared accordingly. Liquid samples can either be used directly or diluted with Assay Buffer and tested. Serum should be diluted approximately 10-100X.Adjust all well volumes to 50 µl.
Note: Pure alcohol is ~17 M. Alcoholic beverages with alcohol in concentrations of 3% (~0.5 M) to 14% (2.4 M) need to be diluted 1000X or more.

3. Initiate Reaction: Each reaction requires 50 µl of reaction mix. Prepare sufficient reaction mix for the total number of wells to be run:

Reaction Mix:

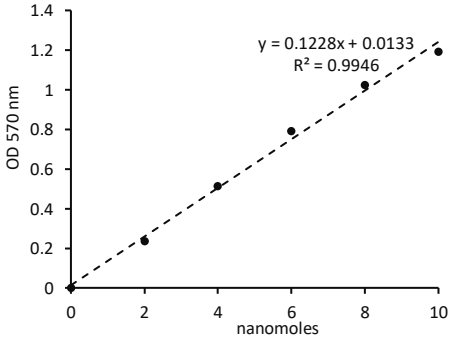
	Absorbance Based Assay	Fluorescence Based Assay
Assay Buffer	46 µl	47.8 µl
ADHP Solution	2 µl	0.2 µl
Alcohol oxidase/Peroxidase	2 µl	2 µl

Add 50 µl of Reaction Mix to wells containing Standards or test samples

4. Measurement: Monitor the reaction at 570 nm (Absorbance based assay) or Excitation 535 nm/Emission 580 nm for 60 min at room temperature.

5. Typical Results:

nmol Standard	Standard Raw Values OD	Background Corrected Values OD
0	0.5112	0
2	0.7498	0.2386
4	1.0269	0.5157
6	1.3042	0.7930
8	1.5354	1.0242
10	1.7037	1.1925



6. Subtract the zero ethanol background value from all other standards and samples (Background signal over ~ 0.1 OD is indicative of trace ethanol in the air. The background reading can be significant and must be subtracted from sample readings. Background of up to 0.5 OD can be tolerated). Plot the Standard Curve. Determine the slope of the Standard Curve. Divide the value of the background corrected Sample values by the slope of the Standard Curve to give nanomoles ethanol in the wells. To convert back to ethanol in the original samples, divide the well value by the volume of sample added to the well = nanomole ethanol per µl of sample. If the sample was derived from tissue or cells, multiply the nanomole ethanol per µl of sample by the total volume of sample and divide by the mg tissue or # of cells used to prepare sample = nanomole per mg tissue (or per # of cells, etc.)

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