AB308327 – Acetaldehyde Assay Kit (Fluorometric)

Determination of acetaldehyde in various sample types.
For research use only - not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit: Abcam Website

Storage and Stability

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

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer XXVIII/ACH Assay Buffer	25 ml	RT
Recombinant ALDH2/ALDH2	50 µl	-20°C
Acetaldehyde/ACH Standard (0.1 M)	0.5 ml	RT
ALDH2 Substrate Mix/ACH Substrate Mix	1 vial	-20 °C
PicoProbe I/ACH Probe (Avoid light)	0.4 ml	RT

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Dounce Tissue Homogenizer
- 96-well white plate with flat bottom
- Multi-well spectrophotometer (Fluorescent plate reader)

Reagent Preparation

Assay Buffer XXVIII/ACH Assay Buffer, Acetaldehyde/ACH Standard (0.1 M) and ACH Probe: Warm to room temperature (RT) before use.

Recombinant ALDH2/ALDH2: Keep on ice until it thaws completely. Divide into aliquots and store at -20 °C. Avoid repeated freeze/thaw cycles.

ACH Substrate Mix: Reconstitute the vial in 220 µl Assay Buffer XXVIII/ACH Assay Buffer. Pipette up and down to dissolve completely. Keep on ice and store at -20 °C.

Non-Enzymatic Cell Harvesting Protocol

Sample Preparation:

For liquid samples (e.g., saliva)

Transfer 0.5 ml of liquid sample into an Eppendorf tube. Centrifuge at 10,000 x g for 20 min at RT. Transfer the supernatant to a new Eppendorf tube. Dilute the sample using Assay Buffer XXVIII/ACH Assay Buffer, if necessary.

For solid or food samples (e.g., banana)

Weigh ~100 mg of sample and transfer to an Eppendorf tube. Add 0.5 ml of cold Assay Buffer XXVIII/ACH Assay Buffer into the tube and homogenize the sample using Dounce tissue homogenizer for 5 min. Centrifuge at 10,000 x g for 20 min at RT. Transfer the supernatant to a new Eppendorf tube. Dilute the sample using Assay Buffer XXVIII/ACH Assay Buffer, if necessary. Add 50 µl of the diluted supernatant into two parallel wells of a 96-well white plate labeled as Sample and Sample Background Control.

Δ Note:

1. *For Unknown Samples, we recommend doing a pilot experiment and testing several doses to ensure that the readings are within the linear range of the Standard Curve.*
2. *Endogenous compounds may interfere with the reaction. To ensure accurate determination of ACH in the test samples, we recommend spiking samples with a known amount of Acetaldehyde/ACH Standard (50 µM).*

Acetaldehyde/ACH Standard Preparation:

Mix 10 µl of 0.1 M Acetaldehyde/ACH Standard with 990 µl Assay Buffer XXVIII/ACH Assay Buffer to generate 1 mM Acetaldehyde/ACH Standard. Further, mix 200 µl of 1 mM Acetaldehyde/ACH Standard with 800 µl of Assay Buffer XXVIII/ACH Assay Buffer to generate 200 µM Acetaldehyde/ACH Standard. Perform a 2-fold serial dilution of 200 µM Acetaldehyde/ACH Standard by mixing 0.5 ml of 200 µM Acetaldehyde/ACH Standard with 0.5 ml of Assay Buffer XXVIII/ACH Assay Buffer to generate 100 µM of Acetaldehyde/ACH Standard. Perform a 2-fold serial dilution of 100 µM Acetaldehyde/ACH Standard and 50 µM Acetaldehyde/ACH Standard to generate 50 µM and 25 µM of Acetaldehyde/ACH Standards respectively. Add 50 µl of 25, 50 and 100 µM of Acetaldehyde/ACH Standards into the desired wells of a 96-well white plate. Add 50 µl Assay Buffer XXVIII/ACH Assay Buffer to the 0 µM Acetaldehyde/ACH Standard well.

Reaction Mix Preparation:

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

	Reaction Mix	Background Mix
Assay Buffer XXVIII/ACH Assay Buffer	45.5 µl	46 µl
Recombinant ALDH2/ALDH2	0.5 µl	---
ACH Substrate Mix	2 µl	2 µl
ACH Probe	2 µl	2 µl

Mix well and add 50 µl of Reaction Mix to all Standard and Sample wells and 50 µl of Background Mix to Sample Background Control wells respectively. Mix well.

Measurement:

Measure the fluorescence at Ex/Em= 535/587 nm in kinetic mode at 25 °C for 30-60 min. The Acetaldehyde/ACH Standard Curve can be read in endpoint mode (at the end of incubation time).

Calculation:

Subtract the 0 Standard reading from all Standard readings and plot the Acetaldehyde/ACH Standard Curve. For Samples and Sample Background Control wells, choose any two time points within the linear range of the curve (t1 & t2) and obtain the corresponding readings (RFU1 and RFU2). Calculate ΔRFUS for Samples, i.e. (ΔRFUS = RFUS2 – RFUS1) and ΔRFUSBC for

Sample Background Control (ΔRFUSBC = RFUSBC2 – RFUSBC1). Then subtract the ΔRFUSBC from ΔRFUS to get the corrected Sample readings. Apply the corrected Sample readings to the Acetaldehyde/ACH Standard Curve to get C µM of ACH. Calculate the ACH concentration (µg/g) in food samples as shown below

ACH (µg/g) = C x V x MW x D / W

Where: **C** is the concentration of ACH from the Standard Curve (in µM)
V is the total sample volume (in L)
MW is the molecular weight of ACH (44 g/mol)
D is the sample dilution factor (if applicable, D = 1 for undiluted samples)
W is the weight of sample used (in g)

Technical Support

For all technical or commercial enquiries please go to:
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