

Version v2b Last updated 6 August 2025

ab272531

Ethanol Assay Kit

View [ab272531](#)

Ethanol Assay Kit datasheet:

www.abcam.com/ab272531

(use www.abcam.cn/ab272531 for China, or www.abcam.co.jp/ab272531 for Japan)

For quantitative determination of Ethanol in alcohol containing samples such as beverages and yeast cultures.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview	1
2. Protocol Summary	2
3. Precautions	3
4. Storage and Stability	3
5. Limitations	4
6. Materials Supplied	4
7. Materials Required, Not Supplied	5
8. Technical Hints	6
9. Reagent Preparation	7
10. Standard Preparation	8
11. Sample Preparation	9
12. Assay Procedure	10
13. Calculations	11
14. Typical Data	12
15. Notes	13

1. Overview

Ethanol Assay Kit (ab275231) is a simple, direct and automation-ready procedure for measuring ethanol concentration. This assay kit is based on an improved dichromate method, in which dichromate is reduced by ethanol to a bluish chromic (Cr^{3+}) product. The intensity of color, measured at 580 nm, is a direct measure of the alcohol concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples and exhibits high sensitivity.

Sensitive and accurate: Detection range 0.04 – 2% alcohol in 96-well plate assay.

Convenient and high-throughput: The procedure involves adding a single working reagent, incubation for 8 min, adding a Stop Reagent, and reading the optical density. Can be readily automated as a high throughput 96-well plate assay for thousands of samples per day.

Versatility: Assays can be executed in 96-well plate or cuvette.

2. Protocol Summary

Prepare all reagents, samples and standard as instructed



Add Samples, and Standard to appropriate tubes.



Add Reagent A to Samples and Standard.



Incubate for 8 to 30 minutes at room temperature.



Add Stop Reagent B to Samples and Standard.



Read absorbance at 580 nm.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store reagents at room temperature and standard at 4°C immediately upon receipt. Avoid multiple freeze-thaw cycles. Kit has a storage time of 12 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Reagent A	50 mL	Room temperature
Reagent B	50 mL	Room temperature
Standard (10% v/v Ethanol)	2 mL	4°C
10% TCA	50 mL	Room temperature

7. Materials Required, Not Supplied

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

- Distilled H₂O
- Multi-channel pipette
- 1.5 mL tubes
- 1.5 mL centrifuge
- 96-well clear plate with flat bottom (alternatively, 1 mL cuvettes may be used)
- Standard microplate reader - capable of reading absorbance at 570-600 nm (peak absorbance is at 580 nm).

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the tube to avoid contamination.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.

9. Reagent Preparation

- Equilibrate reagent to room temperature (18-25°C) prior to use.
- Reagent comes as is ready to use.
- The kit contains enough reagents for 500 assays.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare diluted standards immediately prior to use.

Standard Dilution:

- Procedure using 96-well plate:

10.1.1 Prepare 600 μL of 2% Premix by mixing 120 μL of 10% Standard and 480 μL of dH_2O .

10.1.2 Dilute standard in 1.5 mL centrifuge tubes as described in the table, below.

Standard #	Premix Standard (μL)	dH_2O (μL)	Conc. (%)
1	150	0	2.00
2	120	30	1.60
3	90	60	1.20
4	60	90	0.80
5	45	105	0.60
6	30	120	0.40
7	15	135	0.20
8	0	150	0

- Procedure using cuvette:

10.1.3 Dilute 10% Standard in 1.5 mL centrifuge tubes as described in the table, below.

Standard #	Standard (μL)	dH_2O (μL)	Conc. (%)
A	90	360	2.00
B	45	405	1.00
C	25	475	0.50

11. Sample Preparation

Sample treatment:

- Proteinaceous samples (e.g. culture media)

Deproteination:

11.1 Add 1 vol of sample to 2 vol of 10% TCA.

11.2 Pellet for 5 min at 14,000 rpm on a table centrifuge.

11.3 Carefully transfer supernatant for assay (n = 3).

- Saliva and urine

11.4 Can be analyzed directly (n = 1).

- Wines

11.5 Dilute samples to approximately 1 to 2% prior to assay.

ΔNote: This assay is not suitable for sample containing glucose or glycerol.

ΔNote: For samples containing only sugars (e.g. glucose), remove the interferents prior to assay.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- This assay is based on a kinetic reaction. Addition of Reagent A and B (Stop reagent) should be quick and mixing should be brief but thorough.

Procedure using 96-well plate:

- 12.1.1 Add 100 μ L of standards and samples to separate wells.
- 12.1.2 Add 100 μ L of Reagent A quickly to each well and tap plate lightly to mix.
- 12.1.3 Incubate 8 to 30 min at room temperature.

ΔNote: The reagent color changes from yellow to visibly bluish in wells of Standard #1 to 4.

- 12.1.4 Add 100 μ L of Stop Reagent B quickly to each well and tap plate lightly to mix.
- 12.1.5 Read OD at 570-600 nm (peak 580 nm).

Procedure using cuvette:

- 12.1.6 Add 400 μ L of H₂O (Blank), Standards and samples to appropriately labeled tubes.
- 12.1.7 Add 400 μ L of Reagent A quickly to each well and vortex briefly to mix.
- 12.1.8 Incubate 8 to 30 min at room temperature.
- 12.1.9 Add 400 μ L Reagent B quickly and mix briefly.
- 12.1.10 Transfer to cuvettes.
- 12.1.11 Read OD at 570-600 nm (peak 580 nm).

ΔNote: It is recommended that an interval be applied between additions, e.g., add Reagent A to Tube 1 and 1 min later to Tube 2 etc. After the incubation step is completed, add the Stop Reagent B to Tube 1 and 1 min later to Tube 2 etc. This will ensure identical incubation time between tubes.

13. Calculations

- 13.1.1 Subtract Blank OD (#8 for well-plate procedure) from the Standard.
- 13.1.2 Plot the OD against standard concentrations.
- 13.1.3 Determine sample ethanol concentration from the standard curve.

Δ Note: 1% (v/v) ethanol equals 170 mM or 785 mg/dL.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

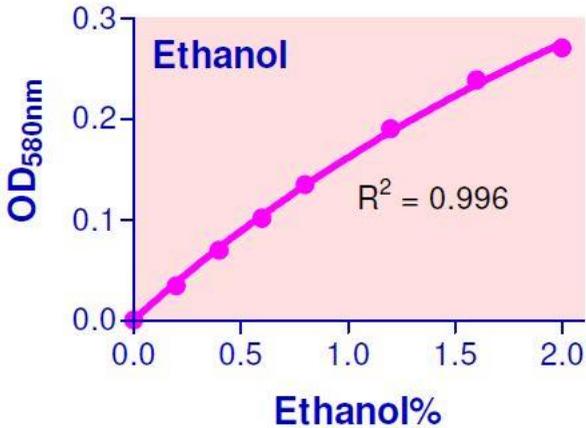


Figure 1. Example of Ethanol Assay Kit standard curve in 96-well plate.

15. Notes

Technical Support

Copyright © 2025 Abcam, All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

For all technical or commercial enquiries please go to:

www.abcam.com/en-us/contact-us

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)