ab65343

Ethanol Assay Kit (Colorimetric/Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of ethanol levels in various samples.

View kit datasheet: www.abcam.com/ab65343
(use www.abcam.cn/ab65343 for China, or www.abcam.co.jp/ab65343 for Japan)

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

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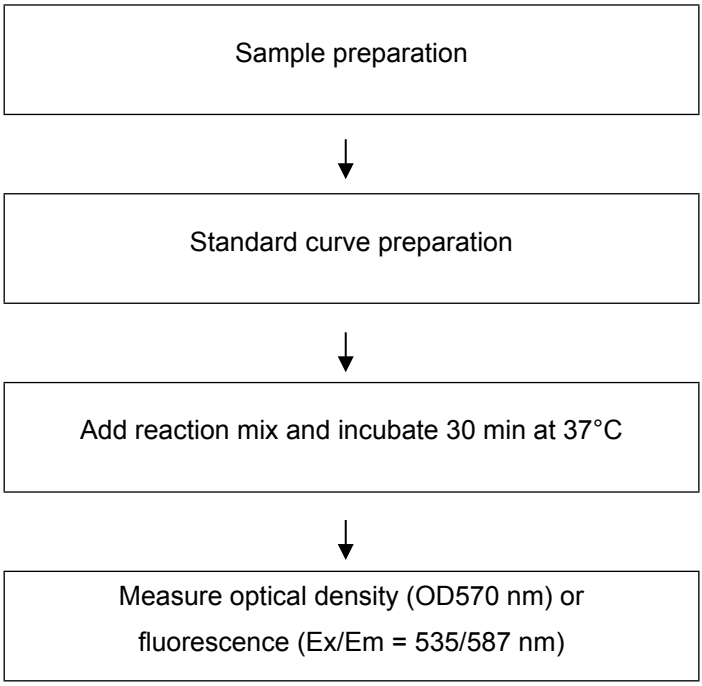
1. BACKGROUND

Ethanol Assay Kit (Colorimetric/Fluorometric) (ab65343) provides a simple, rapid, and sensitive method for accurate quantification of ethanol concentration in a variety of biological samples such as serum, plasma, other body fluids, foods, beverages and growth media. Alcohol oxidase oxidizes ethanol to generate H_2O_2 which reacts with our probe to generate color (λ_{max}= 570 nm) and fluorescence (Ex/Em = 535/587 nm). The kit detects 0.1-10 ppm alcohol (~10-800 µM).

Alcohol (ethanol C_2H_5OH) is among the most widely consumed drinks. Low doses of alcohol may help circulation while heavy alcohol consumption may lead to various forms of disease. Quantitative determination of alcohol finds applications in basic research, drug discovery, clinical studies and fermentation industry processes.

2. ASSAY SUMMARY



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. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Wash Buffer I	25 mL	-20°C	-20°C
OxiRed™ Probe	0.2 mL	-20°C	-20°C
Ethanol Enzyme Mix	1 vial	-20°C	-20°C
Ethanol Standard	0.5 mL	-20°C	-20°C

PLEASE NOTE: Wash Buffer I was previously labelled as Ethanol Assay Buffer, and OxiRed™ Probe as Ethanol Probe (in DMSO, anhydrous), and Ethanol Standard as Ethanol Standard (17.15 N). The composition has not changed.

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent or colorimetric microplate reader – equipped with filter for OD 570 nm or Ex/Em = 535/587 nm (respectively)
- 96 well plate: clear plates for colorimetric assay; black plates (clear bottoms) for fluorometric assay
- Orbital shaker
- Dounce homogenizer (if using tissue)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- 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.

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.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.
- ***NOTE:*** *Extreme care should be taken to ensure that no alcohol vapors (ethanol, methanol, propanol) are in the laboratory air where this assay is to be performed. Alcohol vapors in the air will be rapidly absorbed by kit components resulting in very high background making the kit unusable. Laboratories where HPLC equipment and solvents are standing or where alcohol is used to wipe down laboratory benches or equipment are inappropriate locations to perform this assay.*

9.1 **Wash Buffer I:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C, protect from light and moisture.

9.2 **OxiRed™ Probe:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.** Store at -20°C protected from light. Once the probe is thawed, use with two months.

9.3 **Enzyme Mix:**

Reconstitute with 220 µL Wash Buffer I. Aliquot enzyme mix so that you have enough to perform the desired number of assays. Store at -20°C.

9.4 **Ethanol Standard:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C protected from light and moisture.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 For the colorimetric assay:

10.1.1 Prepare Ethanol Standard by adding 50 µL pure Ethanol Standard to 808.7 µL Wash Buffer I. Mix well.

10.1.2 Prepare 10 nmol/µL Ethanol Standard by adding 10 µL of the dilution to 990 µL Wash Buffer I. Mix well.

10.1.3 Prepare 1 nmol/µL (1 mM) Ethanol Standard by adding 100 µL of the 10nmol/µL dilution into 900 µL Wash Buffer I. Mix well.

10.1.4 Using 1 mM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of 1mM Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End [Ethanol] in well
1	0	150	50	0 nmol/well
2	6	144	50	2 nmol/well
3	12	138	50	4 nmol/well
4	18	132	50	6 nmol/well
5	24	126	50	8 nmol/well
6	30	120	50	10 nmol/well

Each dilution has enough amount of standard to set up duplicate reading (2 x 50 µL).

10.2 For the flurometric assay:

10.2.1 Prepare Ethanol Standard by adding 50 µL of pure Ethanol Standard to 808.7 µL Wash Buffer I. Mix well.

10.2.2 Prepare 10 nmol/µL Ethanol Standard by adding 10 µL of the dilution to 990 µL Wash Buffer I. Mix well.

10.2.3 Prepare 1 mM Ethanol Standard by adding 100 µL of the 10nmol/µL dilution into 900 µL Wash Buffer I. Mix well.

10.2.4 Prepare 0.1 mM Ethanol Standard by diluting 100 µL of 1 mM Ethanol Standard to 900 µL of Assay Buffer.

10.2.5 Using 0.1mM Ethanol Standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of 0.1mM Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End [Ethanol] in well
1	0	150	50	0 nmol/well
2	6	144	50	0.2 nmol/well
3	12	138	50	0.4 nmol/well
4	18	132	50	0.6 nmol/well
5	24	126	50	0.8 nmol/well
6	30	120	50	1.0 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

NOTE: *If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.*

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 **Serum and biological fluid samples:**

Samples can be diluted in Assay Buffer for testing. Biological samples such as serum (containing ~0.01-0.16% w/v) should be diluted 1:10-1:100 and volumes in the range of 10 – 30 µL used.

11.2 **For beverages:**

Samples can be diluted in Assay Buffer for testing. Beverages which contain 100X more alcohol, correspondingly greater dilutions should be used.

Adjust the final volume to 50 µL using Wash Buffer I.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 10 – 30 µL samples (adjust volume to 50 µL/well with Assay Buffer).
- Background wells = 50 µL Wash Buffer I.

12.2 **Reaction Mix:**

Prepare 50 µL of Reaction Mix for each reaction:

Component	Colorimetric Reaction Mix (µL)	Fluorometric Reaction Mix (µL)*
Wash Buffer I	**46**	**47.8**
OxiRed™ Probe*	**2**	**0.2**
Ethanol Enzyme Mix	**2**	**2**

NOTE: **For fluorometric assay, use 0.2 µL OxiRed™ Probe per well to reduce background.*

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + standards +1).

12.3 Add 50 µL of the Reaction Mix to all wells.

12.4 Mix and incubate at 37°C for 30 minutes, or at room temperature for 60 minutes, protected from light.

12.5 Measure output on a microplate reader.

- Colorimetric assay: measure OD 570 nm.
- Fluorometric assay: measure Ex/Em = 535/587 nm.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.3 Plot the corrected absorbance values for each standard as a function of the final concentration of ethanol.

13.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.5 Extrapolate sample readings from the standard curve plotted using the following equation:

$$Sa = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

13.6 Concentration of samples in the test samples is calculated as:

$$Ethanol\ Concentration = \frac{Sa}{Sv}$$

Where:

Sa = Sample amount from the Standard Curve (nmol).

Sv = Sample volume added into the sample well (µL).

Ethanol molecular weight: 46.07 g/mol.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

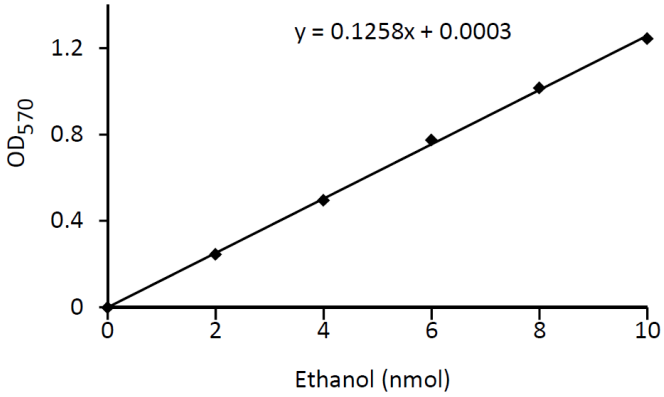


Figure 1: Typical Ethanol standard calibration curve using colorimetric reading.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Solubilize Ethanol Enzyme Mix, thaw OxiRed™ Probe, Ethanol Standard and Wash Buffer I (aliquot if necessary); get equipment ready
- Prepare appropriate standard curve for your detection method of choice (colorimetric or fluorometric).
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL) and samples (50 µL).
- Prepare Ethanol Reaction Mix (Number samples + standards + 1).

Component	Colorimetric Reaction Mix (µL)	Fluorometric Reaction Mix (µL)
Wash Buffer I	**46**	**47.8**
OxiRed™ Probe*	**2**	**0.2**
Ethanol Enzyme Mix	**2**	**2**

- Add 50 µL Ethanol Reaction Mix to each well.
- Incubate plate at RT for 60 mins or at 37°C for 30 mins.
- Measure plate at OD 570nm for colorimetric assay or Ex/Em= 535/587 nm for fluorometric assay.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of inappropriate plate for reader	Colorimetry: Clear plates Fluorescence: Black plates (clear bottom)
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer (increase number of strokes); observe for lysis under microscope
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so as to be in the linear range

17. FAQs

Can the initial dilution of the standard prep curve for the colorimetric assay (50 µL pure ethanol standard to 808.7 µL Wash Buffer I) be aliquoted and frozen for future use?

It is not recommended to freeze the pure Ethanol standard because some ethanol might vaporize changing the concentration of the solution during thawing. The pure ethanol standard should be prepared fresh ideally.

A flat standard curve is obtained but the values are very high. Zero ethanol standard curve point shows high values.

This indicates there has been methanol/ethanol/propanol absorption into the buffer or other components. This is a classic diagnostic feature when the zero standard shows high values and due to saturation, the standard curve is flat. Extreme care should be taken to perform this assay in places where alcohol has not been used on the benches or there is no chance of contamination in the air. The fluorometric assay being at least 10 times more sensitive, can show larger interference due to contamination. Even incubating samples with ethanol next to blank might sometimes result in cross-contamination.

What kind of anti-coagulants should be used for blood/plasma/serum sample type used for this kit?

Heparin can be used with this kit.

Why is there pink color in the reaction mix? Isn't color development only possible when there is sample/ethanol?

The probe is colorless to begin with. But it is very sensitive to oxygen and moisture in the air. It is possible that the probe has been exposed to oxygen/water and has oxidized before adding it to the alcohol-containing samples. This might explain the pink color seen. Also, this kit is very sensitive to methanol/ethanol/propanol vapor in the lab and the probe can react to this vapor and yield a pink color.

What is the recommended way to prepare and store the enzyme mix i.e. do you vortex the enzyme mix or gently mix by inversion? Do you keep enzyme mix on ice or at room temperature? Should I use cold assay buffer to dissolve enzyme mix?

On the day of the experiment, if all 96/100 samples + standards are to be run together, the assay buffer (warmed to room temperature) can be used to reconstitute the enzyme mix. The enzyme mix can be vortexed and used for the assay immediately. If the enzyme mix is to be used multiple times, the reconstituted enzyme can be stored at +4°C for up to 2 months. Alternately, the enzyme mix can be reconstituted in cold assay buffer and kept on ice while the samples are being aliquoted into the wells. The reaction with the samples will occur at room temperature or 37°C during the incubation in any case.

18. INTERFERENCES

These chemicals or biological will cause interferences in this assay causing compromised results or complete failure.

- Traces of ethanol in the atmosphere.
- EDTA.

19. FAQs

Q. Will this kit detect Isopropanol?

No, the kit is specific for ethanol as the enzyme used cannot detect isopropanol.

Q. What is the exact volume of sample required for this assay?

The kit detects 0.1-10 ppm alcohol (~10-800 nM) in samples. Hence depending upon the amount of ethanol in the samples, the volume needs to be optimized such that readings are within the linear range of the standard curve.

Q. Can protein content be used as an internal control for this assay?

Yes, a detergent-compatible BCA assay can be used for protein quantitation to normalize sample amount.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

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