



## **Ethanol Assay Kit (Colorimetric)**

Ethanol Assay Kit (Colorimetric) is a detection kit for the quantification of Ethanol in Alcoholic beverages.

Catalog number: ARG82152

Package: 100 tests

---

For research use only. Not for use in diagnostic procedures.

## **TABLE OF CONTENTS**

<b>SECTION</b>	<b>Page</b>
INTRODUCTION.....	3
PRINCIPLE OF THE ASSAY.....	3
MATERIALS PROVIDED & STORAGE INFORMATION.....	4
MATERIALS REQUIRED BUT NOT PROVIDED.....	4
TECHNICAL NOTES AND PRECAUTIONS.....	5
SAMPLE COLLECTION & STORAGE INFORMATION.....	6
REAGENT PREPARATION.....	7
ASSAY PROCEDURE.....	7
CALCULATION OF RESULTS.....	8
EXAMPLE OF TYPICAL STANDARD CURVE.....	8
QUALITY ASSURANCE.....	9

**MANUFACTURED BY:**

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: [info@arigobio.com](mailto:info@arigobio.com)

### INTRODUCTION

Ethanol (also called ethyl alcohol, grain alcohol, drinking alcohol, or simply alcohol) is an organic chemical compound. It is a simple alcohol with the chemical formula  $C_2H_6O$ . Its formula can be also written as  $CH_3-CH_2-OH$  or  $C_2H_5OH$  (an ethyl group linked to a hydroxyl group), and is often abbreviated as EtOH. Ethanol is a volatile, flammable, colorless liquid with a slight characteristic odor. It is a psychoactive drug, recreational drug, and the active ingredient in alcoholic drinks.

Ethanol is naturally produced by the fermentation of sugars by yeasts or via petrochemical processes such as ethylene hydration. It has medical applications as an antiseptic and disinfectant. It is used as a chemical solvent and in the synthesis of organic compounds. Ethanol is a fuel source. [Provide by Wikipedia: Ethanol]

### PRINCIPLE OF THE ASSAY

This Ethanol Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of Ethanol present in alcohol containing samples such as beverages. This assay 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 O.D. 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.

## Ethanol Assay Kit (Colorimetric) ARG82152

---

### MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on room temperature. Store reagents at room temperature and the ethanol standard at 4°C. Shelf life: 12 months after receipt.

Component	Quantity	Storage information
Reagent A	50 mL	RT
Reagent B	50 mL	RT
10% TCA	50 mL	RT
Standard (10% v/v, Ethanol)	2 mL	4°C

### MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 580 nm
- Clear flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

### TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- If sample contains glucose or glycerol, use arigobio ethanol assay kit (ARG82151).
- 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.
- Proteinaceous samples (E.g., plasma, serum, culture media, should be deproteinated by adding 1 vol sample to 2 vol 10% TCA). Centrifugation for 5 minutes at 14,000 rpm, carefully transfer supernatant for assay.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

### **SAMPLE COLLECTION & STORAGE INFORMATION**

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

**Serum:** Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collect the serum and assay directly.

**Plasma:** Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collect the plasma layer and assay directly.

**Saliva and urine:** Assay directly.

**Beverages:** dilute samples to approximately 1 to 2% prior to assay.

#### **Note:**

- Proteinaceous samples (E.g., plasma, serum, culture media, should be deproteinated by adding 1 vol sample to 2 vol 10% TCA). Centrifugation for 5 minutes at 14,000 rpm, carefully transfer supernatant for assay.
- If sample contains glucose or glycerol, use arigobio ethanol assay kit (ARG82151).
- Dilute factor: Serum / Plasma n=3; Saliva / urine n=1.

## Ethanol Assay Kit (Colorimetric) ARG82152

---

### REAGENT PREPARATION

- **Standard:** Prepare 600  $\mu\text{L}$  of 2% Premix by mixing 120  $\mu\text{L}$  of 10% Standard and 480  $\mu\text{L}$  of distilled water. Dilute standard as follows.

Standard tube	Ethanol (%)	Distilled water ( $\mu\text{L}$ )	Standard Premix ( $\mu\text{L}$ )
S1	2.0	0	150
S2	1.6	30	120
S3	1.2	60	90
S4	0.8	90	60
S5	0.6	105	45
S6	0.4	120	30
S7	0.2	135	15
S0	0	150	0

### ASSAY PROCEDURE

Equilibrate reagents to room temperature. Briefly centrifuge tubes before use.

	Standard well	Sample well
Standard	100 $\mu\text{L}$	
Sample		100 $\mu\text{L}$
Add <b>100 <math>\mu\text{L}</math></b> of <b>Reagent A</b> into each well quickly. Tap plate lightly to mix.		
Tap plate to mix briefly and thoroughly. Incubate for <b>8-30 minutes</b> at <b>room temperature</b> . The reagent color changes from yellow to visibly bluish.		
Add <b>100 <math>\mu\text{L}</math></b> of <b>Reagent B</b> into each well quickly. Tap plate to mix.		
Read the absorbance at <b>O.D. 580 nm</b> .		

**Note:** Addition of Reagent A and B should be quick and mixing should be brief but thorough. Use of multi-channel pipettor is recommended.

## Ethanol Assay Kit (Colorimetric) ARG82152

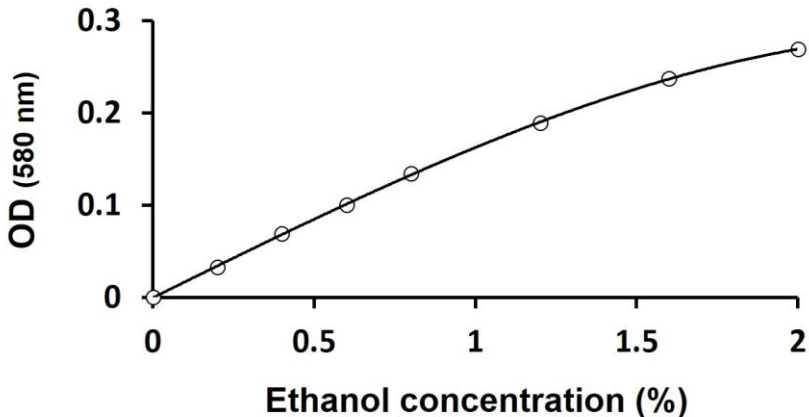
---

### CALCULATION OF RESULTS

1. Subtract blank (distilled water, S8) from the Standards OD values and plot the OD against standard alcohol concentrations. Determine sample ethanol concentration from the standard curve.
2. Conversions: 1% (v/v) ethanol equals 170 mM or 785 mg/dL.
3. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure.

### EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Ethanol Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.





**QUALITY ASSURANCE**

**Sensitivity**

0.04%