Ethanol Content Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: NA0178

Size: 100T/96S

Components:

Reagent I: Liquid 15μL×1, store at -20℃. According to the required amount of the experiment, according to the ratio of reagent I: distilled water =0.5 μL: 49.5 μL (5T amount), mix well, and prepare before use.

Reagent II : Liquid 10 mL×1, store at 2-8℃.

Reagent III A: Liquid 5mL×1, store at 2-8℃.

Reagent III B: Liquid 5mL×1, store at 2-8℃. According to the amount required in the experiment, in accordance with the ratio of reagent III A: reagent III B = 1:1, mix well, and prepare before use.

Standard: Liquid 0.5mL×1, store at 2-8℃. Before use, mix 50 μL of standard and 350 μL of distilled water to prepare a standard solution of 2.14 mol/L before use.

Product Description：

Wine is a general term for alcoholic (ethanol) beverages, and ethanol is the main component of wine and one of the important indicators to measure the quality of wine. Ethanol can be used in the manufacture of acetic acid, beverages, flavors, dyes, fuels, etc. Ethanol with a volume fraction of 70% to 75% is commonly used as a disinfectant in medicine. Ethanol has a wide range of uses in the chemical industry, medical and health, food industry, agricultural production and other fields.

Ethanol is oxidized under the catalysis of alcohol oxidase to produce hydrogen peroxide. Peroxidase catalyzes the oxidation of hydrogen peroxide to 4-aminoantipyrine to couple phenol to generate a colored compound with a characteristic absorption peak at 505nm. The change of the absorption peak at 505nm can be measured to reflect the ethanol content.

Reagents and Equipment Required but Not Provided：

Spectrophotometer/microplate reader, desk centrifuge, constant temperature incubator/water bath, pipette, micro glass cuvette/96 well plate, mortar/homogenizer, ice and distilled water.

Procedure

I. Sample preparation:

1. **Tissue sample:** According to the proportion of tissue weight (g): distilled water (mL) of 1:5-10 to extract. It is suggested that 0.1 g of tissue with 1 mL of distilled water and fully homogenized on ice bath. Centrifuge at 8000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.
2. **Liquid sample:** Detect directly. If the liquid is cloudy, the supernatant can be collected after centrifugation.

II. Determination procedure：

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 505 nm, set spectrophotometer counter to zero with distilled water.
2. Add reagents with the following list:

Reagent（μL）	Test tube(t)	Standard tube(s)	Blank tube(b)
Reagent I	10	10	10
Reagent II	90	90	90
Reagent III	90	90	90
Sample	10	-	-
Standard	-	10	-
Distilled water	-	-	10

Mix thoroughly，immediately measure the absorbance value A1 at 505nm, then put the cuvette and the reaction solution in 37℃ (mammal) or 25℃ (other species) water bath for 60 minutes, take it out and wipe it clean and immediately determine its the absorbance value A2 in 60min. Calculate ΔAt = A2t-A1t, ΔAs = A2s-A1s, ΔAb = A2b-A1b. Blank tube and standard tube only need to test once or twice.

If the number of samples is too large, reagent I, reagent II, and reagent III can be prepared into working solution in proportion.

III. Calculations：

1. Sample weight

Ethanol Content (mmol /g weight)＝（ΔAt-ΔAb）×C÷（ΔAs-ΔAb）×Vs÷(Vs÷Ve×W) ×F

＝（ΔAt-ΔAb）×2.14÷（ΔAs-ΔAb）÷W×F

2. Liquid volume

Ethanol Content (mmol / L) ＝（ΔAt-ΔAb）×C÷（ΔAs-ΔAb）×F×1000

＝（ΔAt-ΔAb）÷（ΔAs-ΔAb）×F×2140

V_S：Add sample volume, 0.01 mL；

V_E: Extract solution volume, 1 mL;

W: Sample weight, g;

C：Standard tube concentration，2.14 mmol/mL;

F: Dilution ratio;

1000: Unit conversion factor, 1mL=0.001L.

Note:

1. If the measured absorbance valueΔA>0.5, it is recommended to dilute the sample before measuring, and multiply the dilution factor in the calculation formula; if the measured absorbance value is low or close to the blank OD value, it is recommended to increase the sample volume before performing the measurement.
2. If the number of samples is too large, reagent I, reagent II, and reagent III can be prepared into working solution in proportion.

3. It’s better not to test too many samples to avoid affecting enzymatic reaction time.

Experimental example:

1. Take 0.1g mice liver, add 1 mL of distilled water, grind the homogenate with ice bath. Then operate according to the determination steps, calculate ΔAt=A2t-A1t = 0.086-0.06=0.026, ΔAs = A2s-A1s=0.630-0.053=0.577, ΔAb = A2b-A1b=0.057-0.048=0.009. The result is calculated according to the sample weight:

   Ethanol Content (mmol /g weight)＝（ΔAt-ΔAb）×2.14÷（ΔAs-ΔAb）÷W×F =0.052 mmol /g weight

2. Take 10 μL of perfume, operate according to the determination steps, calculate ΔAt=A2t-A1t = 0.989-0.166=0.823, ΔAs = A2s-A1s=0.630-0.053=0.577, ΔAb = A2b-A1b=0.057-0.048=0.009. The result is calculated according to liquid volume:

   Ethanol Content (mmol /L)＝（ΔAt-ΔAb）÷（ΔAs-ΔAb）×F×2140 =3066.8 mmol /L

Related Products：

NA0808/NA0566 Aldehyde Dehydrogenase(ALDH) Activity Assay Kit
NA0790/NA0549 Alcohol Dehydrogenase (ADH) Activity Assay Kit
NA0712/NA0471 Lactic Acid(LA) Content Assay Kit