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: BC6035

Size:100T/96S

Components:

Extraction reagent I: Liquid 110 mL×1. Store at 2-8℃.

Extraction reagent II: Liquid 20 mL×1. Store at 2-8℃

Reagent I: Liquid 12 mL×1. Store at 2-8℃.

Reagent II: Powder ×1. Store at -20℃, Dissolve with 1.2mL of distilled water before use. It can be stored at -20℃ for 4 weeks. Avoid repeated freeze-thaw.

Reagent III: Powder ×1. Store at -20℃, Dissolve with 1.5mL of distilled water before use. It can be stored at -20℃ for 4 weeks. Avoid repeated freeze-thaw.

Reagent IV: Liquid 4 mL×1. Store at 2-8℃.

Reagent V: Liquid 0.4 mL×1. Store at -20℃.

Reagent V working liquid: Before use according to Reagent V: distilled water =1μL: 99μL (Total 0.1mL, about 40T) proportion dilution Reagent V.

Standard goods: Liquid 0.5 mL×1. Store at 2-8℃.

1**.25μmol/mL Standard solution**: Before use, 58.4μL standard solution was taken, and 941.6μL distilled water was added to mix thoroughly to prepare 1000μmol/mL standard solution. Then take 25μL 1000μmol/mL standard solution, add 975μL distilled water, mix thoroughly to prepare 25μmol/mL standard solution. Then take 50μL 25μmol/mL standard solution, add 950μL distilled water, mix thoroughly to prepare 1.25μmol/mL standard solution for the determination of the following standard tubes.

Product Description

Alcohol is the general name of alcoholic (ethanol) beverages, ethanol is the main component of alcohol, is one of the important indicators to measure the quality of wine. Ethanol can be used to manufacture acetic acid, beverages, flavors, dyes, fuels, etc. In medical treatment, ethanol with a volume fraction of 70% to 75% is commonly used as a disinfectant. Ethanol is widely used in chemical industry, medical care, food industry, agricultural production and other fields.

Ethanol dehydrogenase catalyzes the dehydrogenation of ethanol to acetaldehyde, while reducing NAD^+ to produce NADH and H^+. Under the action of 1-mPMS, WST-1 can react with NADH to produce water-soluble formazan, which has a maximum absorption peak at 450nm, according to which the ethanol content can be calculated.

Reagents and Equipment Required but Not Provided.

Spectrophotometer/Microplate reader, mortar/homogenizer/cell ultrasonic crusher, centrifuge,

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constant temperature foster box/water-bath, micro quartz cuvette/96 well flat-bottom plate, ice and distilled water.

Procedure:

I. Sample preparation

1. **Tissue:** According to the proportion of tissue weight (g): Extraction reagent I volume (mL) of 1:5-10 to extract. It is suggested that 0.1 g of tissue with 1 mL of Extraction reagent I and fully homogenized
on ice bath. Centrifuge at 12000 g for 10 minutes at 4℃. Take 0.8mL of supernatant, add 0.15mL of Extraction reagent II slowly blow and mix until there is no bubble, centrifuge at 4℃ at 12000g for 10 minutes, take the supernatant on ice before testing.
2. **Cells/Bacteria:** Collect cells into the centrifuge tube, discard supernatant after centrifugation. According to the proportion of cells number (10^4): Extraction reagent I volume (mL) of 500-1000:1 to extract. It is suggested that 5 million of cell amount with 1 mL of Extraction reagent I. Split the cell with ultrasonication (placed on ice, ultrasonic power 200W, working time 3s, interval 7s, Total times:5 minutes). Centrifuge at 12000 g for 10 minutes at 4℃. Take 0.8mL of supernatant, add 0.15mL of Extraction reagent II slowly blow and mix until there is no bubble, centrifuge at 4℃ at 12000g for 10 minutes, take the supernatant on ice before testing.
3. **Serum (plasma):** Take 100μL Serum (plasma) and add 1mL Extraction reagent I, centrifuge at 4℃ at 12000g for 10min, take 0.8mL supernatant, and then add 0.15mL Extraction reagent II, slowly blow and mix until there is no bubble, centrifuge at 4℃ at 12000g for 10min, take the supernatant on ice before testing.
4. **Other liquids:** direct measurement. If there is turbidity, centrifuge and take supernatant to be measured.
   Note: The Extraction reagent II needs to be added slowly, and a large number of bubbles will be generated after addition. It is recommended to use 2mL EP tube for operation.

II. Determination

1. Preheat the spectrophotometer or microplate reader for more than 30 minutes, adjust the wavelength to 450 nm and set spectrophotometer counter to zero with distilled water.
2. Preheat the Reagent I at 37℃ for more than 10min before use.
3. Sample Test (add Reagent in the micro quartz cuvette /96 well flat-bottom plate):

Reagent（μL）	Test tube (T)	Standard tube (S)	Blank tube (B)
Sample	40	-	-
Standard goods	-	40	-
distilled water	-	-	40
Reagent I	100	100	100
Reagent II	10	10	10
Reagent III	10	10	10

Reagent IV	30	30	30
Reagent V working liquid	10	10	10

After full mixing, the absorption value A1 at 450nm for 15s is determined, and the reaction is quickly placed in a 37℃ water bath or constant temperature incubator for 10min (the temperature control function of the enzyme spectrometer can be adjusted to 37℃). The absorption value A2 of 10min15s is determined and recorded as $A1_S$, $A1_T$, $A1_B$ and $A2_S$, $A2_T$, $A2_B$. Calculation $\Delta A_T = (A2_T - A1_T) - (A2_B - A1_B)$; $\Delta A_S = (A2_S - A1_S) - (A2_B - A1_B)$. The standard tube and blank tube only need to be measured 1-2 times.

III. Calculation of Ethanol content:

1. Sample weight:

   Ethanol content（μmol/g weight）$= \Delta A_T \times C_S \div \Delta A_S \times (V_{SV}+V_{EVII}) \div (W \times V_{SV} \div V_{EVI}) \times F$
   $=1.484 \times \Delta A_T \div \Delta A_S \div W \times F$

2. Protein concentration:

   Ethanol content（μmol/mg prot）$= \Delta A_T \times C_S \div \Delta A_S \times V_S \div (V_S \times Cpr) \times F = 1.25 \times \Delta A_T \div \Delta A_S \div Cpr \times F$

3. Cell/Bacteria amount:

   Ethanol content（μmol/10^4 cell）$= \Delta A_T \times C_S \div \Delta A_S \times (V_{SV}+V_{EVII}) \div (N \times V_{SV} \div V_{EVI}) \times F$
   $=1.484 \times \Delta A_T \div \Delta A_S \div N \times F$

4. Calculated by Serum (plasma) volume:

   Ethanol content（μmol/mL）$= \Delta A_T \times C_S \div \Delta A_S \times (V_{SV}+V_{EVII}) \div [V_{LS} \times V_{SV} \div (V_{EVI}+ V_{LS})] \times F$
   $=16.328 \times \Delta A_T \div \Delta A_S \times F$

5. According to the volume of other liquids:

   Ethanol content（μmol/mL）$= \Delta A_T \times C_S \div \Delta A_S \times V_S \div V_S \times F$
   $=1.25 \times \Delta A_T \div \Delta A_S \times F$

C_S: Standard tube concentration, 1.25μmol/mL.
V_S: Sample volume,0.1 mL.
V_{SV}: Supernatant volume,0.8 mL.
V_{EVI}: Extraction volume I, 1 mL.
V_{EVII}: Extraction volume II, 0.15 mL.
V_{LS}: Serum (plasma) sample volume at the time of extraction,0.1 mL.
W: Sample quality, g.
N: Number of cells/bacteria, measured as 10^4.
F: Sample dilution ratio.

Note:

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1. To ensure the accuracy and stability of the experimental results, please strictly control the reaction time and operation time. It is not recommended to determine too many samples at one time, so as not to

affect the consistency of enzymatic reaction time.
2. If the absorption value of the Test tube ΔA>0.8 or A1>1.5, it is recommended to dilute the sample
with distilled water before measuring, and pay attention to the simultaneous modification of the calculation formula.
3. If the absorption value ΔA of the Test tube<0.01, it is recommended to increase the sample size or increase the sample proportion in the sample processing step before testing. If the sample size is increased, the blank tube and standard tube need to be adjusted accordingly.
4. The extraction solution contains protein precipitator. If it is necessary to calculate the protein concentration, it needs to be extracted again to determine the protein concentration.

Experimental examples:

1. Take 10μL beer and dilute it with distilled water for 500 times, then follow the determination procedure. After determination with 96 well flat-bottom plate, calculate $\Delta A_T = (A1_T - A1_T) - (A1_B - A1_B)$ = (0.682-0.096) -(0.107-0.058) =0.537. $\Delta A_S = (A1_S - A1_S) - (A1_B - A1_B)$ = (0.573-0.070) - (0.107- 0.058) =0.454, To calculate:
   Ethanol content（μmol/mL）=1.25×ΔAT÷ΔAS×F = 739.26 μmol/mL

References:

[1] Bergmeger H U. Methods of enzymatic analysis, 3rd, Vol III[M]. Verlag Chemse: Weinhein Press, 1983: 598-616.

[2] Ivory R, Delaney E, Mangan D. et al. Determination of Ethanol Concentration in Kombucha Beverages: Single-Laboratory Validation of an Enzymatic Method [J]. Journal of AOAC INTERNATIONAL, 2021, 104(2): 422-430.

Related Products：

BC0750/BC0755 Aldehyde Dehydrogenase(ALDH) Activity Assay Kit
BC1080/BC1085 Alcohol Dehydrogenase (ADH) Activity Assay Kit
BC2230/BC2235 Lactic Acid(LA) Content Assay Kit

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