Histamine 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: BC5675

Size: 100T/48S

Components:

Extraction reagent I: Liquid 60 mL×1. Store at 2-8℃.

Extraction reagent II: Liquid 10 mL×1. Store at 2-8℃

Reagent I: Liquid 25 mL×1. Store at 2-8℃.

Reagent II: Powder ×1. Store at 2-8℃, The first reagent is dissolved in 65μL **distilled water**. It can be stored at 2-8℃ for 4 weeks.

Reagent II diluent: Liquid 2.5 mL×1. Store at 2-8℃.

Preparation of Reagent II working liquid: Before use according to Reagent II: **Reagent II diluent** =10μL: 300μL (Total 310μL, About 10S) proportion. Use it now.

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

Powder I: Powder 3g ×1. Store at 2-8℃.

Standard goods: Liquid 1 mL×1. Store at 2-8℃. 10μmol/mL Histamine Standard solution.

0.1875μmol/mL standard preparation: Before use, take 75μL 10μmol/mL Histamine standard solution was taken, and 925μL distilled water was added to mix thoroughly to prepare 0.75μmol/mL Histamine standard solution. Then take 250μL 0.75μmol/mL Histamine standard solution, add 750μL distilled water, mix thoroughly to prepare 0.1875μmol/mL Histamine standard solution for use.

Product Description

Histamine is a potentially harmful nitrogen containing low molecular weight organic compound, which is produced by histidine under the action of decarboxylase. Histamine is released when tissue is damaged or when inflammation and allergic reactions occur. Histamine is widely found in various foods, and excessive intake of histamine will cause harm to human body.

Histamine is specifically broken down by histamine dehydrogenase (HDH), and under 1-mPMS, electron transfer occurs through WST for color rendering, with a maximum absorption peak at 470nm, according to which histamine content can be calculated

Reagents and Equipment Required but Not Provided.

Spectrophotometer/microplate reader, mortar/homogenizer/cell ultrasonic crusher, centrifuge, constant temperature foster box/water-bath, micro glass cuvette/96 well flat-bottom plate, ice and distilled water.

Procedure:

I. Sample preparation

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1. **Tissue:** According to the proportion of tissue weight (g): Extraction reagent I volume (mL) of 1:2.5-5 to extract. It is suggested that 0.2 g of tissue with 1 mL of Extraction reagent I and fully homogenized on ice bath. It was bathed in water at 60℃ for 30min, cooled to room temperature and centrifuged at 4℃ 10000g for 10minutes. Take 0.8mL of supernatant, then slowly add 0.15mL of Extraction reagent II, slowly blow and mix until no bubbles emerge. Centrifuge at 4℃ at 12000g for 10 minutes, take the supernatant on ice before testing.
2. **Red wine and other liquids (high phenolic content) sample:** Weigh Powder I 0.05g, take 500μL liquid sample and add 500μL Extraction reagent I. After the ice bath is homogenized, it is bathed in water at 60℃ for 30minutes, cooled to room temperature, and centrifuged at 4℃ 12000g for 10minutes. Take 0.8mL of supernatant, then slowly add 0.15mL of Extraction reagent II, slowly blow and mix until no bubbles emerge. Centrifuge at 4℃ at 12000g for 10 minutes, take the supernatant on ice before testing.

Note:

1. 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.
2. After sample extraction, the determination should be completed within 2 hours as far as possible. If the sample size is too large, it is recommended to process in batches.
3. For samples with high phenolic content, Powder I ~ 0.05g was added during pre-treatment, and the sample was homogenized in ice bath together. For example, for red wine, take 500μL liquid sample and add 500μL Extraction reagent I, and Powder I about 0.05g (no need to accurately weigh) during pre-treatment, and then continue with follow-up treatment such as ice bath homogenization according to the liquid sample.

II. Determination

1. Preheat the spectrophotometer for more than 30minutes, adjust the wavelength to 470 nm and set spectrophotometer counter to zero with distilled water.
2. Reagent I at 37℃ for 15 minutes.
3. Sample Test (add Reagent in the 1.5mL EP tube/96 well flat-bottom plate):

Reagent（μL）	Test tube (T)	Control tube(C)	Blank tube (B)	Standard tube (S)
Reagent I	195	225	195	195
Reagent II working liquid	30	-	30	30
Reagent III	45	45	45	45
distilled water	-	-	30	-
Sample	30	30	-	-
Standard goods	-	-	-	30

Mix thoroughly, Light avoidance reaction at 37℃ for 15minutes, read the absorbance of wavelength at 470 nm. Note the light absorption values of blank tube, standard tube, test tube and control tube as A_B, A_S, A_T, Ac, and A_C respectively. Calculation $\Delta A_T = A_T - Ac$; $\Delta A_S = As - A_B$. The standard tube and blank tube only need to be measured 1-2 times.

III. Calculation of Histamine content:

1. Protein concentration:

   Histamine content（μmol/mg prot）$= \Delta A_T \times C_S \div \Delta A_S \times V_S \div (V_S \times Cpr) \times F = 0.1875 \times \Delta A_T \div \Delta A_S \div Cpr \times F$

   C_S: Standard tube concentration, 0.1875μmol/mL;

   V_S: Sample volume, 0.03mL;

   Cpr: Sample protein concentration, mg/mL; The protein concentration should be determined by oneself.

   F: Dilution ratio.

2. Sample weight:

   Histamine content（μmol/g weight）$= \Delta A_T \times C_S \div \Delta A_S \times (V_{SV}+V_{EVII}) \div (W \times V_{SV} \div V_{EVI}) \times F$
   $= 0.223 \times \Delta A_T \div \Delta A_S \div W \times F$

   C_S: Standard tube concentration, 0.1875μmol/mL;

   W: Sample quality, g;

   V_{SV}: supernatant volume,0.8 mL;

   V_{EVI}: Extraction volume I, 1 mL;

   V_{EVII}: Extraction volume II, 0.15 mL;

   F: Dilution ratio.

3. Volume of liquid:

   Histamine content（μmol/mL）$= \Delta A_T \times C_S \div \Delta A_S \times (V_{SV}+V_{EVII}) \div [V_{LS} \times V_{SV} \div (V_{EVI+} V_{LS})] \times F$
   $= 0.445 \times \Delta A_T \div \Delta A_S \times F$

   C_S: Standard tube concentration, 0.1875μmol/mL;

   V_{SV}: supernatant volume,0.8 mL;

   V_{EVI}: Extraction volume I, 0.5 mL;

   V_{EVII}: Extraction volume II, 0.15 mL;

   V_{LS}: Liquid sample volume, 0.5 mL;

   F: Dilution ratio.

Note:

1. Extraction reagent I contains a protein precipitator, so the supernatant cannot be used for protein concentration determination. For determination of protein content, separate tissue should be taken.
2. If the measured absorbance value of the sample is less than 0.05 or close to the absorbance value of the blank tube, the sample size can be appropriately increased. The blank tube and the standard tube should also be adjusted accordingly, and the calculation formula should be modified simultaneously.
3. When the $\Delta A_T>1$ or $A_T>1.5$, the sample can be diluted with distilled water for determination.

Related Products:

BC0010/BC0015	Monoamine Oxidase Activity Assay Kit
BC1280/BC1285	Diamine Oxidase (DAO) Activity Assay Kit
BC5220/BC5225	Polyamine Oxidase (PAO) Activity Assay Kit

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