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CheKine™ Micro Hydroxyproline (HYP) Content Assay Kit

Cat #: KTB1490 Size: 96 T

	Micro Hydroxyproline (HYP) Content Assay Kit		
REF	**Cat #:** KTB1490	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal Tissue, Bacteria or Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

HYP is one of the main components of collagen in the body. Most of the collagen is distributed in the skin, tendon, cartilage and blood vessels et al. Therefore, the content of HYP is an important index reflecting the metabolism and fibrosis degree of collagen tissue. CheKine™ Micro Hydroxyproline (HYP) Content Assay Kit can be used to detect biological samples such as animal tissue, bacteria or cells, serum or plasma. In the kit, the sample is hydrolyzed to produce free HYP, which is further oxidized by chloramine T. The oxidized product reacted with p-Dimethylaminobenzaldehyde to produce red compound with characteristic absorption peak at 560 nm. The content of HYP can be calculated by measuring the absorption value of sample hydrolysate at 560 nm.

Materials Supplied and Storage Conditions

Kit components	Size (96 T)	Storage conditions
Cell Extraction Buffer	100 mL	4℃
Reagent Ⅰ	6 mL	4℃, protected from light
Reagent Ⅱ	6 mL	4℃, protected from light
Standard	0.5 mL	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 560 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube, Glass tube
- Water bath, centrifuge machine, Baking oven
- Deionized water, Concentrated hydrochloric acid, Ethanol anhydrous, Isopropyl alcohol

Reagent Preparation

Tissue Extraction Buffer: Ready to use as supplied; 6 mol/L hydrochloric acid, concentrated hydrochloric acid: deionized water

(V/V)=1:1; Store at room temperature. **(Required but not provided)**

Cell Extraction Buffer: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent I: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Reagent II: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Standard： Ready to use as supplied; 0.5 mg/mL hydroxyproline. Equilibrate to room temperature before use; Store at 4℃, protected from light.

Standard preparation: Use the 0.5 mg/mL hydroxyproline standard solution and further dilute it to the standard as shown in the following table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (μg/mL)
Std.1	24 μL 0.5 mg/mL Standard	376	30
Std.2	200 μL of Std.1 (30 μg/mL)	200	15
Std.3	200 μL of Std.2 (15 μg/mL)	200	7.5
Std.4	200 μL of Std.3 (7.5 μg/mL)	200	3.75
Std.5	200 μL of Std.4 (3.75 μg/mL)	200	1.875
Std.6	200 μL of Std.5 (1.875 μg/mL)	200	0.938
Std.7	200 μL of Std.6 (0.938 μg/mL)	200	0.469
Std.8	200 μL of Std.7 (0.234 μg/mL)	200	0.234

Notes: Always prepare fresh Standards per use; Diluted Std. solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Animal Tissues: Weigh 0.2 g tissue in a glass tube, add 2 mL Tissue Extraction Buffer, placed in an baking oven at 110℃, hydrolyzed for 6-12 h, fixed volume to 2 mL with Tissue Extraction Buffer. Centrifuge at 12,000 g for 20 min at 25℃. Use supernatant for assay.
2. Bacteria or Cells: Collect 5×10^6 bacteria or cells into the centrifuge tube, add 1 mL Cell Extraction Buffer. After keeping 30 min at 15 pounds in a high pressure disinfector and lowering the pressure naturally, centrifuge at 12,000 g for 20 min at 25℃. Use supernatant for assay.
3. Extraction of serum free HYP: Take 0.1 mL serum, add 0.5 mL anhydrous ethanol to precipitate the protein centrifuge at 8,000 g for 5 min at 4℃. Pour the supernatant into another EP tube, blow with nitrogen or take a boiling water bath to evaporate the ethanol. Add 0.2 mL 50% isopropanol after cooling, fully dissolve and mix well to be tested.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 560 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement. (The following operations are operated in the 1.5 mL EP tube)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Sample	0	0	60

Standard	0	60	0
Reagent Ⅰ	60	60	60
Mix well and let stand at room temperature for 20 min.			
Reagent Ⅱ	60	60	60
Deionized water	180	120	120

3. Mix thoroughly, 20 min was reacted at 60℃, and 15 min was placed at room temperature after being removed. Add 200 μL to microglass cuvette/96 well plate, detect the absorbance at 560 nm after cooling with running water. The Blank Well is recorded as A_{Blank}, the standard Well is marked as $A_{Standard}$, the Test Well is marked as A_{Test}. Finally calculate $=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Blank Well and the Standard Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.02, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.8, the sample can be appropriately diluted with corresponding Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve and obtain the standard equation y=kx+b. The determination of ΔA_{Test} is brought into the equation to get x (μg/mL).

2. Calculation of the HYP content

(1) Calculated by sample protein concentration

HYP (μg/mg prot)=$x\times V_{Sample}\div(Cpr\times V_{Sample})$**=x÷Cpr**

(2) Calculated by fresh weight of samples

HYP (μg/g fresh weight)=$x\times V_{Reaction\ volume}\div(V_{Sample}\div V_{Total\ sample}\times W)$**=10x÷W**

(3) Calculated by bacteria or cells

HYP (μg/10^4)=$x\times V_{Reaction\ volume}\div(V_{Sample}\div V_{Total\ sample}\times n)$**=5x÷n**

(4) Calculated by volume of liquid samples

HYP (μg/mL)=$x\times V_{Reaction\ volume}\div(V_{Sample}\div 2)$**=10x**

$V_{Reaction\ volume}$: Total volume of reaction, 0.3 mL; V_{Sample}: Added sample volume to the reaction system, 0.06 mL; $V_{Total\ sample}$: Added the Extraction Buffer volume, mL; Cpr: Sample protein concentration, mg/mL; W: Sample weight, g; n: Number of bacteria or cells, ten thousand; 2: Multiple of re-dissolution of serum after blow-drying.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

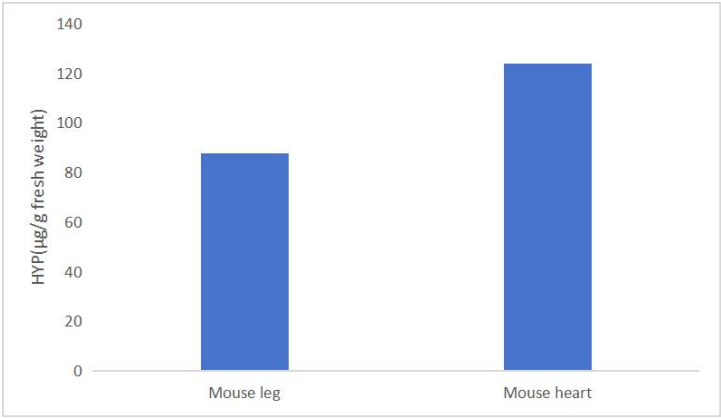

Figure 1. Determination of HYP content in mouse leg and heart by this kit.

Recommended Products

Catalog No.	Product Name
KTB1410	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1420	CheKine™ Micro Aspartate Aminotransferase (AST/GOT) Activity Assay Kit
KTB1430	CheKine™ Micro Proline (PRO) Content Assay Kit
KTB1440	CheKine™ Micro Glutamate (Glu) Content Assay Kit
KTB1450	CheKine™ Micro Cysteine (Cys) Content Assay Kit
KTB1460	CheKine™ Micro Amino Acid (AA) Content Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.