Hydroxyproline (HYP) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Detection equipment: Spectrophotometer

Catalog Number: BC0250

Size: 50T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Self-supplied reagent	-
Reagent I	Liquid 15 mL×1	2-8℃
Reagent II	Liquid 15 mL×1	2-8℃
Standard	Liquid 1 mL×1	2-8℃

Solution Preparation:

1. Extract solution: 6 mol/L hydrochloric acid (HCl), self-provided reagent, about 100mL, stored at room temperature; An empty brown 30mL bottle is provided in the kit. Please label the reagent name by yourself.

2. 6 mol/L hydrochloric acid (HCl): Concentrated HCl (37%): H_2O (V/V) =1:1, stored at room temperature.

3. Standard: 0.5 mg/mL hydroxyproline.

Product Description:

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.

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.

Technical Specifications:

Minimum Detection limit:0.051 μg/mL

Linear Range: 0.117-7.5 μg/mL

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment. If the absorption value of the sample is not within the measurement range, it is recommended to dilute or increase the sample size for detection.

Reagents and Equipment Required but Not Provided:

Scales, glass tube, desk centrifuge, water bath, visible spectrophotometer, 1 mL glass cuvette, 6 mol/L

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HCl, Sodium hydroxide solution and distilled water.

Operation procedure:

I. Sample preparation (The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1. Tissue sample:

Weigh about 0.2 g of the sample into the glass tube, cut the tissue into pieces as much as possible for digestion. Add 2 mL of Extract solution and the cover is slightly loose and not airtight, boil it or bake it in 110℃ oven for 2 to 6 hours to digest it until there is no visible big lump (Wrap the sealing film to prevent bursting).

After cooling, adjust the pH to 6-8 with 10 mol/L NaOH (Do not over acid or over alkali) then constant volume to 4 mL with distilled water. Centrifugation at 16000 rpm for 20 minutes at 25℃ (if there is still impurity after centrifugation, it can be removed by filtration).

Take the supernatant for test.

2. Bacteria or cell:

Take 5 million bacteria/cells, add 1 mL of Extract solution, boil or oven at 110℃ for 2 to 6 hours to digest to transparent state(Wrap the sealing film to prevent bursting).

After cooling, adjust the pH to 6-8 with 10 mol/L NaOH (Do not over acid or over alkali) then constant volume to 2 mL with distilled water. Centrifugation at 16000 rpm for 20 minutes at 25℃ (if there is still impurity after centrifugation, it can be removed by filtration).

Take the supernatant for test.

3. Liquid sample:

Take a 300μL liquid sample in a glass tube. Add 0.7 mL of Extract solution (If the measured value is too small, the ratio of the two can be adjusted), boil it or bake it in 110℃ oven for 2 to 6 hours to digest it until there is no visible big lump (Wrap the sealing film to prevent bursting.)

After cooling, adjust the pH to 6-8 with 10 mol/L NaOH (Do not over acid or over alkali) then constant volume to 2 mL with distilled water. Centrifugation at 16000 rpm for 20 minutes at 25℃ (if there is still impurity after centrifugation, it can be removed by filtration).

Take the supernatant for test.

Note: Black substance may be formed in the process, and if it cannot be digested for a long time, it may be carbonized substance. It does not affect the experiment.

II. Determination procedure:

1. Preheat spectrophotometer for more than 30 minutes, adjust the wavelength to 560 nm, set zero with distilled water.

2. Standard preparation: Dilute the standard solution to 15, 7.5, 3.75, 1.875, 0.938, 0.469, 0.234, 0.117 μg/mL with distilled water.

3. Operation table:

Reagent (μL)	Blank tube (B)	Test tube (T)	Standard tube (S)
Sample	-	200	-
Standard	-	-	200
Reagent I	200	200	200
Mix well and leave it at room temperature for 20 minutes.			

Reagent II	200	200	200
distilled water	600	400	400
Mix well, incubate at 60℃ for 20 minutes, let stand at room temperature for 15 minutes. Use 1mL glass cuvette to detect the absorbance value of each tube at 560 nm, $\Delta A_T=A_T-A_B$, $\Delta A_S=A_S-A_B$. (The blank tube and standard curve only need to be measured 1-2 times.)			

III. Calculation:

1. Making of standard curve.

When making the standard curve, the concentration of the standard solution is taken as the x-axis, and the ΔA_S($\Delta A_S=A_S-A_B$) is taken as the y-axis. The linear equation y=kx+b is obtained. Take ΔA_T ($\Delta A_T=A_T-A_B$) to the equation to acquire x.

2. Calculation of hydroxyproline content:

(1) Calculate by sample mass

Hydroxyproline content (μg/g mass) = $x\times V_S\div(W\times V_S\div V_{TE})\times F = 4x\div W\times F$.

(2) Calculate by sample protein concentration:

Hydroxyproline content (μg/mg prot) = $x\times V_S\div(Cpr\times V_S)\times F = x\div Cpr\times F$.

(3) Calculate by the number of bacteria or cells:

Hydroxyproline content (μg/10^6 cell) = $x\times V_S\div(N\times V_S\div V_{CE})\times F = 2x\div N\times F$.

(4) Calculate by liquid volume

Hydroxyproline content (μg/mL) = $x\times V_{LE}\div V_L\times F = 6.67\times x\times F$.

V_S: Volume of added sample, 0.2 mL;
V_{TE}: Volume of tissue extract solution, 4 mL;
V_{CE}: Volume of cell extract solution, 2 mL;
V_{LE}: Volume of liquid extract solution, 2 mL;
V_L: Volume of liquid added in pre-treatment, 0.3mL;
W: Fresh weight of sample, g;
N: Number of cells, 10^6 as a unit;
Cpr: Concentration of sample protein, mg/mL.

Note

1. If the measured light absorption value exceeds the linear range of light absorption value, you can increase the sample size or dilute the sample with distilled water before the determination Pay attention to
multiply the dilution multiple in the calculation formula.
2. The reagent has certain toxicity. Please take protective measures during operation to prevent inhalation or contact with skin.
3. When calculating according to the sample protein concentration, the protein in the sample itself needs to be extracted separately and determined.

Experimental examples：

1.Take 0.2 g of mouse skin and add 1mL extract for sample pretreatment, take the supernatant and dilute it with distilled water for 32 times and follow the determination steps. The $\Delta A_T=A_T-A_B$= 0.548-0.124 =0.424 measured by 1 mL glass cuvette, and the standard curve y=0.0634x +0.0044, R^2=0.9996 is brought in, and x = 6.62 is calculated.

Hydroxyproline content (μg/g mass) = 4x ÷ W×F = 4×6.62÷0.2×32=4236.8 μg/g mass.

2. Take 8×10^6 HMC-1 cells and add 1mL extract for sample pretreatment, take the supernatant and follow the determination steps. The $\Delta A_T=A_T-A_B$= 0.245-0.124 =0.121 measured by 1 mL glass cuvette, and the standard curve y=0.0634x +0.0044, R^2=0.9996 is brought in, and x = 1.84 is calculated.

Hydroxyproline content (μg/10^6 cell) =2x÷N×F =2×1.84÷8=0.46 μg/10^6 cell.

3. Take 300μL Bovine serum and add 0.7mL extract for sample pretreatment, take the supernatant and follow the determination steps. The $\Delta A_T=A_T-A_B$= 0.243-0.124 =0.119 measured by 1 mL glass cuvette, and the standard curve y=0.0634x +0.0044, R^2=0.9996 is brought in, and x = 1.81 is calculated.

Hydroxyproline content (μg/mL) = 6.67×x×F = 6.67×1.81=12.07 μg/mL.

Recent Product Citations:

[1] Litong Fan,Jiaqing Chen,Yanmeng Tao,et al. Enhancement of the chondrogenic differentiation of mesenchymal stem cells and cartilage repair by ghrelin. Journal of Orthopaedic Research. January 2019;(IF3.043).

[2] Qu P, Rom O, Li K, Jia L, Gao X, Liu Z, Ding S, Zhao M, Wang H, Chen S, Xiong X, Zhao Y, Xue C, Zhao Y, Chu C, Wen B, Finney AC, Zheng Z, Cao W, Zhao J, Bai L, Zhao S, Sun D, Zeng R, Lin J, Liu W, Zheng L, Zhang J, Liu E, Chen YE. DT-109 ameliorates nonalcoholic steatohepatitis in nonhuman primates. Cell Metab. 2023 May 2;35(5):742-757.e10. doi: 10.1016/j.cmet.2023.03.013. Epub 2023 Apr 10. PMID: 37040763.

[3] Hao Y, Zhou J, Tan J, Xiang F, Qin Z, Yao J, Li G, Yang M, Zeng L, Zeng W, Zhu C. Preclinical evaluation of the safety and effectiveness of a new bioartificial cornea. Bioact Mater. 2023 Aug 8;29:265-278. doi: 10.1016/j.bioactmat.2023.07.005. PMID: 37600931; PMCID: PMC10432718.

References:

[1] Naeini A, Miri R, Shafiei N. et al. Effects of topical application of Calendula officinalis gel on collagen and hydroxyproline content of skin in rats[J]. Comparative Clinical Pathology, 2012, 21: 253-257.

[2] Ignat'eva N, Danilov N, Averkiev S. et al. Determination of hydroxyproline in tissues and the evaluation of the collagen content of the tissues[J]. Journal of Analytical Chemistry, 2007, 62: 51-57.

Related Products:

BC1550/BC1555 Glutamic-pyruvic Transaminase (GPT) Activity Assay Kit

BC1560/BC1565 Glutamic-oxalacetic Transaminase (GOT) Activity Assay Kit

BC0290/BC0295	Proline (PRO) Content Assay Kit
BC1570/BC1575	Amino Acid (AA) Content Assay Kit
BC0180/BC0185	Cysteine (Cys) Content Assay Kit
BC1580/BC1585	Glutamic Acid (Glu) Content Assay Kit

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