Hydroxyproline (HYP) Activity Assay Kit

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

Detection equipment: Spectrophotometer/Microplate reader

Cat No: BC0255

Size: 100T/96S

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 8 mL×1	2-8℃
Reagent II	Liquid 8 mL×1	2-8℃
Standard	Liquid 0.5 mL×1	2-8℃

Solution Preparation:

1. Extract solution: 6 mol/L hydrochloric acid (HCl), self-provided reagent, about 200mL, 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:0.057 μg/mL

Linear Range: 0.234-30 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.

Required but not provided:

Scales, oven, glass tube, centrifuge, water bath, spectrophotometer/microplate reader, micro glass cuvette/96-well plate, 6 mol/L HCl, sodium hydroxide solution and distilled water.

Operation procedure:

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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/microplate reader for 30 minutes, adjust wavelength to 560 nm and set zero with distilled water.
2. Dilute the 0.5 mg/mL hydroxyproline standard solution to 30, 15, 7.5, 3.75, 1.875, 0.938, 0.469, 0.234 μg/mL.
3. Add reagents as the following table.

Reagent (μL)	Blank tube (B)	Test tube (T)	Standard tube (S)
Sample	-	60	-
Standard	-	-	60
Reagent I	60	60	60
Mix well and leave it at room temperature for 20 minutes.			
Reagent II	60	60	60

distilled water	180	120	120
Mix well, incubate at 60℃ for 20 minutes, let stand at room temperature for 15 minutes. Use micro glass cuvette/96 well flat-bottom plate 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(A_T-A_B) to the equation to acquire x.

1. Calculation of hydroxyproline content:

(1) calculated according to the fresh weight of the sample:

Tissue hydroxyproline content (μg/g fresh weight) = $x\times V_S\div(W\times V_S\div V_{TE})\times F = 4x\div W\times F$.

(2) calculated according to the sample protein concentration:

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

(3) calculated according to the number of bacteria or cells:

Cell hydroxyproline content (μg/10^4 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, 0.06 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 OD value is greater than 1.0, the sample should be diluted properly and then determined. 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 $\Delta A_T=A_T-A_B=0.375-0.123=0.252$

measured by 96-well plate, and the standard curve y=0.0344x+0.015, R^2=0.9975 is brought in, and x = 6.89 is calculated.

Hydroxyproline content (μg/g mass) = 4x ÷ W×F = 4×6.89÷0.2×32=4409.6 μg/g weight

2. Take $8×10^6$ HMC-1 cells and add 1mL extract for sample pretreatment, take the supernatant and follow the determination steps. The $\Delta A_T=A_T-A_B$=0.183-0.126=0.057 measured by 96-well plate, and the standard curve y=0.0344x+0.015, R^2=0.9975 is brought in, and x = 1.22 is calculated.

Hydroxyproline content (μg/10^6 cell) =2x÷N=2×1.22÷8=0.305 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 $\Delta A_T=A_T-A_B$=0.194-0.123=0.071 measured by 96-well plate, and the standard curve y=0.0344x+0.015, R^2=0.9975 is brought in, and x = 1.63 is calculated.

Hydroxyproline content (μg/mL) = 6.67×x×F = 6.67×x=6.67×1.63=10.87 μg/mL.

Recent Product Citations:

[1] Zhao P, Sun T, Lyu C, Liang K, Niu Y, Zhang Y, Cao C, Xiang C, Du Y. Scar-Degrading Endothelial Cells as a Treatment for Advanced Liver Fibrosis. Adv Sci (Weinh). 2023 Feb;10(4):e2203315. doi: 10.1002/advs.202203315. Epub 2022 Dec 9. PMID: 36494102; PMCID: PMC9896053.

[2] Chen G, An N, Shen J, Chen H, Chen Y, Sun J, Hu Z, Qiu J, Jin C, He S, Mei L, Sui Y, Li W, Chen P, Guan X, Chu M, Wang Y, Jin L, Kim K, Li X, Cong W, Wang X. Fibroblast growth factor 18 alleviates stress-induced pathological cardiac hypertrophy in male mice. Nat Commun. 2023 Mar 4;14(1):1235. doi: 10.1038/s41467-023-36895-1. PMID: 36871047; PMCID: PMC9985628.

[3] Wu K, Liu Y, Xia J, Liu J, Wang K, Liang H, Xu F, Liu D, Nie D, Tang X, Huang A, Chen C, Tang N. Loss of SLC27A5 Activates Hepatic Stellate Cells and Promotes Liver Fibrosis via Unconjugated Cholic Acid. Adv Sci (Weinh). 2024 Jan;11(2):e2304408. doi: 10.1002/advs.202304408. Epub 2023 Nov 13. PMID: 37957540; PMCID: PMC10787101.

[4] Xia S, Liu Z, Cai J, Ren H, Li Q, Zhang H, Yue J, Zhou Q, Zhou T, Wang L, Liu X, Zhou X. Liver fibrosis therapy based on biomimetic nanoparticles which deplete activated hepatic stellate cells. J Control Release. 2023 Mar;355:54-67. doi: 10.1016/j.jconrel.2023.01.052. Epub 2023 Feb 2. PMID: 36693527.

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

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