TECHNICAL MANUAL

Hydroxyproline (HYP) Colorimetric Assay Kit

- **SKU CODE:** MAES0211
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- ![img-0.jpeg](img-0.jpeg)

2. Intended use

This kit can measure hydroxyproline (HYP) content in serum, animal tissue, and urine samples.

3. Detection principle

The sample is hydrolyzed to generate free HYP, and hydroxyproline can produce an oxidation product under the action of an oxidizing agent. The generated oxidation product can react with a chromogenic agent to produce burgundy color. The concentration of hydroxyproline can be calculated by measuring the OD value at 558 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Oxidant Agent	Powder x 1 vial	Powder x 1 vial	2-8°C, 12 months, shading light
Reagent 2	Buffer Solution	8 mL x 1 vial	15 mL x 1 vial	2-8°C, 12 months
Reagent 3	Oxidant Agent Solvent	8 mL x 1 vial	15 mL x 1 vial	2-8°C, 12 months
Reagent 4	Chromogenic Agent	Powder x 1 vial	Powder x 1 vial	2-8°C, 12 months, shading light
Reagent 5	Chromogenic Agent Solvent	28 mL x 1 vial	52 mL x 1 vial	2-8°C, 12 months
Reagent 6	HYP Standard	5 mg x 1 vial	5 mg x 2 vials	2-8°C, 12 months, shading light
Reagent 7	pH Adjusting Solution A	60 mL x 1 vial	60 mL x 2 vials	2-8°C, 12 months
Reagent 8	pH Adjusting Solution B	60 mL x 1 vial	60 mL x 2 vials	2-8°C, 12 months
Reagent 9	Clarificant	Powder x 1 vial	Powder x 2 vials	2-8°C, 12 months

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Vortex mixer, Centrifuge, Water bath, Microplate reader (550-570 nm, optimum wavelength: 558 nm)

Reagents:

2 mol/L Hydrochloric acid, 6 mol/L Hydrochloric acid, Concentrated hydrochloric acid (12 mol/L), N-propyl alcohol.

6. Reagent preparation

Size 1(48 T):

1. Equilibrate all reagents to room temperature before use.

2. The preparation of Oxidant working solution:

Dissolve one vial of oxidant agent with 6 mL of oxidant agent solvent, mix well to dissolve. Add 6 mL of buffer solution, mix well to dissolve. Store at 2-8°C for 5 days protected from light.

3. The preparation of Chromogenic working solution:

Dissolve one vial of chromogenic agent with 25 mL of chromogenic agent solvent. Mix well to dissolve. Store at 2-8°C for 5 days protected from light.

4. The preparation of 1 mg/mL HYP standard:

Dissolve one vial of HYP Standard with 5 mL of double distilled water. Mix well to dissolve. Store at 2-8°C for 15 days.

5. The preparation of 100 μg/mL HYP standard:

Dilute 40 μL of 1 mg/mL HYP standard with 360 μL of double distilled water, mix well. The 100 μg/mL HYP standard should be prepared fresh.

6. The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 100 μg/mL standard solution with double distilled water to serial concentrations. The recommended dilution gradient is as follows: 0, 1, 2, 3, 4, 6, 8, 10 μg/mL.

Standard curve preparation table:

Item: ①②③④⑤⑥⑦⑧

Concentration (μg/mL): 0, 1, 2, 3, 4, 6, 8, 10

100 μg/mL standard solution (μL): 0, 10, 20, 30, 40, 60, 80, 100

Double distilled water (μL): 1000, 990, 980, 970, 960, 940, 920, 900

Size 2(96 T):

1. Equilibrate all reagents to room temperature before use.

2. The preparation of oxidant working solution:

Dissolve one vial of oxidant agent with 12 mL of oxidant agent solvent, mix well to dissolve. Add 12 mL of buffer solution, mix well to dissolve. Store at 2-8°C for 5 days protected from light.

3. The preparation of Chromogenic working solution:

Dissolve one vial of chromogenic agent with 50 mL of chromogenic agent solvent. Mix well to dissolve. Store at 2-8°C for 5 days protected from light.

4. The preparation of 1 mg/mL HYP standard:

Dissolve one vial of HYP Standard with 5 mL of double distilled water. Mix well to dissolve. Store at 2-8°C for 15 days.

5. The preparation of 100 μg/mL HYP standard:

Dilute 40 μL of 1 mg/mL HYP standard with 360 μL of double distilled water, mix well. The 100 μg/mL HYP standard should be prepared fresh.

6. The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 100 μg/mL standard solution with double distilled water to serial concentrations. The recommended dilution gradient is as follows: 0, 1, 2, 3, 4, 6, 8, 10 μg/mL.

Standard curve preparation table:

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Concentration (μg/mL): 0, 1, 2, 3, 4, 6, 8, 10

100 μg/mL standard solution (μL): 0, 10, 20, 30, 40, 60, 80, 100

Double distilled water (μL): 1000, 990, 980, 970, 960, 940, 920, 900

7. Sample preparation

Sample preparation

Tissue and urine sample:

Tissue sample hydrolysis: Accurately weigh 100 mg tissue sample, cut into pieces and put into a glass tube, add 1 mL of 6 mol/L hydrochloric acid, seal and hydrolyze at 95°C for 6 h.

Urine sample hydrolysis: Take 0.5 mL of urine sample into a glass tube, add 0.5 mL of concentrated hydrochloric acid (12 mol/L), seal and hydrolyze at 95°C for 6 h.

Adjust the pH value of sample hydrolysate: Cool sample hydrolysate with running water, and add 0.5 mL of pH Adjusting Solution A and 0.25 mL of pH Adjusting Solution B and mix fully, then add pH Adjusting Solution B drop by drop. Measure the pH value of the solution to 6.5-7.0 using precision pH test paper. If the pH exceeds 7.0, it can be adjusted to 6.5-7.0 with 2 mol/L hydrochloric acid. Add double distilled water to a final volume of 10 mL and mix fully.

Decolorization of sample hydrolysate: Take 2 mL sample hydrolysate into the centrifugal tube, add about 20 mg of clarificant and mix fully, centrifuge at 1500×g for 10 minutes, then take the supernatant for detection.

Serum sample:

Mix 200 µL of serum sample with 800 µL of n-propanol fully, centrifuge at 4°C at 8000×g for 10 min, and supplement the supernatant with double distilled water to 1 mL for detection.

Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type - Dilution factor:

10% Rat liver tissue homogenate - 1

10% Rat kidney tissue homogenate - 1

10% Rat lung tissue homogenate - 1

10% Rat brain tissue homogenate - 1

Chicken Tendon - 20-30

Fish scale - 20-30

Porcine cartilage - 15-25

Human Urine - 1

Note: The diluent is double distilled water (For small tissue samples, the addition of hydrochloric acid solution, pH adjustment solution and final constant volume can be reduced proportionally. At least 400 µL of sample hydrolysate is required for detection).

For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. Strictly control reaction time and temperature.

2. Adjust the pH value to 6.5-7.0 after sample hydrolysis.

3. Cut the tissue samples. It is recommended to prepare the samples one day in advance when samples are limited, and they can be stored at 2-8°C after adjusting pH and volume.

9. Operating steps

1. Standard tube: Take 400 µL of standard solution with different concentrations to the 2 mL EP tube. Sample tube: Take 400 µL of sample to the 2 mL EP tube.

2. Add 200 µL of oxidant working solution to each tube.

3. Mix fully and stand at room temperature for 15 min.

4. Add 400 µL of chromogenic working solution to each tube.

5. Mix fully and incubate the tubes at 60°C for 15 min.

6. Cool the tubes to room temperature with running water, then take 200 µL to the corresponding wells of microplate.

7. Measure the OD value of each well at 558 nm with microplate reader.

10. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard # ①) from all standard readings. This is the absolute OD value.

3. Plot the standard curve by using absolute OD value of standard and corresponding concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

1. Tissue sample:

HYP content (µg/mg wet weight) = (ΔA - b) ÷ a × V ÷ m × f

2. Urine sample:

HYP content (μg/mL) = (ΔA - b) ÷ a × V ÷ V_1 × f

3. Serum sample:

HYP content (μg/mL) = (ΔA - b) ÷ a × V_3 ÷ V_2 × f

[Note]

ΔA: OD_{Sample} – OD_{Blank}.

V: The volume of sample hydrolysate after pH adjustment, 10 mL.

f: Dilution factor of sample before test.

m: The weight of the sample, mg.

V_1: The volume of urine sample, mL.

V_2: The volume of serum sample, mL.

V_3: The final volume of supernatant of serum sample, mL.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μg/mL)	1.20	3.50	7.80
%CV	1.5	1.1	1.0

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μg/mL)	1.20	3.50	7.80

Parameters	Sample 1	Sample 2	Sample 3
%CV	4.2	4.3	4.7

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with each concentration analyzed 6 times in parallel to obtain an average recovery rate of 101%.

Recovery

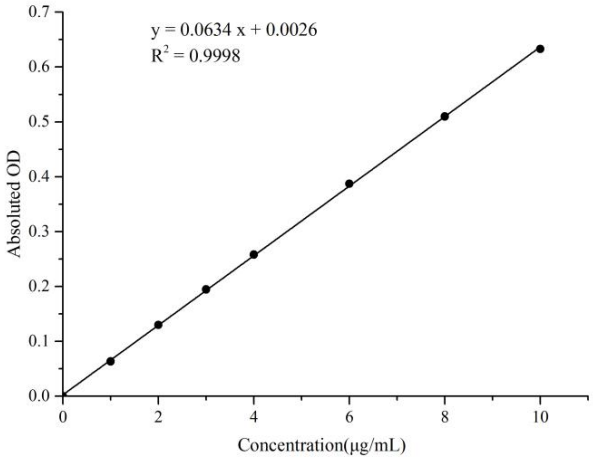
	standard 1	standard 2	standard 3
Expected Conc. (µg/mL)	1.6	3.8	7.4
Observed Conc. (µg/mL)	1.6	3.8	7.7
recovery rate(%)	99	100	104

Sensitivity

The analytical sensitivity of the assay is 0.04 µg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided below for reference only:



Standard curve

Concentration (μg/mL)	Average OD	Absolute OD
0	0.068	0.000
1	0.131	0.063
2	0.194	0.126
3	0.257	0.189
4	0.320	0.252
6	0.446	0.378
8	0.572	0.504
10	0.698	0.630

12. Appendix II Example Analysis

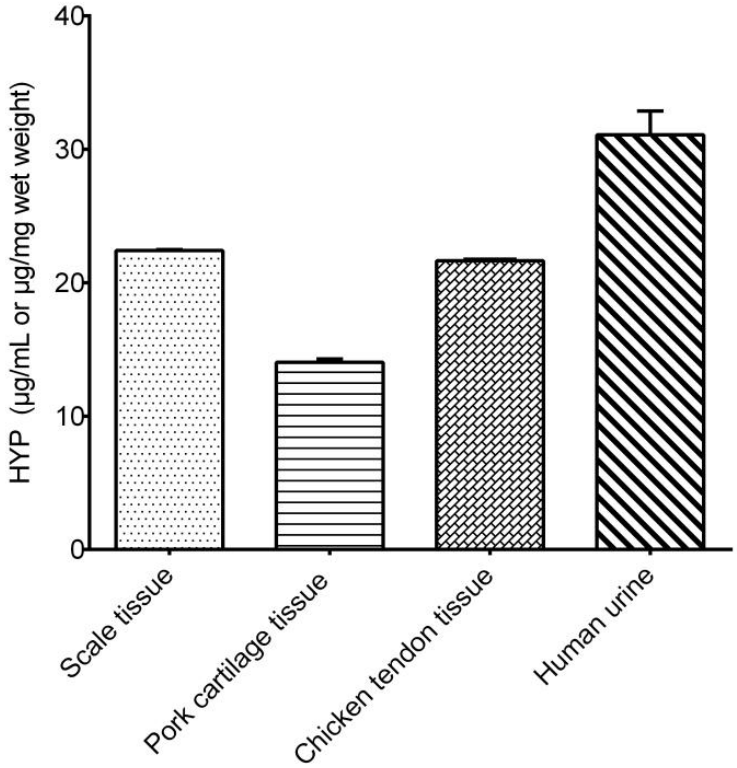
Example analysis:

For fish scale, weigh 98.3 mg fish scale sample, take the hydrolyzed sample and dilute 25 times, and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0615x + 0.0035, the average OD value of the blank is 0.069, the average OD value of the sample is 0.615, and the calculation result is:

HYP content (μg/mg wet weight) = (0.615 – 0.069 – 0.0035) ÷ 0.0615 × 10 × 25 ÷ 98.3 = 22.43 μg/mg wet weight

Detect fish scale (dilute 25 times), porcine cartilage (dilute 20 times), chicken tendon (dilute 25 times) and human urine according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.