Hsphasetin (HP) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/ Microplate Reader

Catalog Number: BC4345

Size: 100T/48S

Components:

Reagent I: Liquid 20mL×1, store at 4℃.

Reagent II: Liquid 3mL×1, store at 4℃.

Reagent III: Powder×1, store at 4℃. Add 6 mL of distilled water to dissolve the reagent before use. Unused reagent is still stored at 4℃ for four weeks.

Standard: Liquid 1mL ×1, store at 4℃. 9 μmol/mL Fe^{2+} standard solution.

Product Description:

Hsphasetin (HP) is a homologue of ceruloplasmin, which catalyzes the oxidation of ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}), then Fe^{3+} binds to transferrin and participates in cellular iron release.

With Fe^{2+} as substrate, Fe^{2+} is oxidized to Fe^{3+} under the catalysis of HP. Fe^{2+} forms a colored complex with phenazine, and has a characteristic absorption peak at 562 nm. The content of Fe^{2+} which is not oxidized is calculated, and then the content of oxidized Fe^{2+} is obtained. So the HP activity can be reflected by the rate at Fe^{2+} oxidized.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, adjustable transferpettor, balance, mortar/homogenizer, centrifuge, micro glass cuvette/ 96 well flat-bottom plate and distilled water.

Sample preparation:

1. Plant and animal tissues: Plant and animal tissues: mass (g): the volume of distilled water (mL) is 1: 5 ~ 10, weigh about 0.1 g of sample, add 1 mL of distilled water, Ice bath homogenate and fully grind. Centrifuge 10000 rpm at 4°C for 10 min, Take the supernatant on ice for testing.
2. Serum or culture medium: It is recommended to dilute serum or plasma 2-4 times with distilled water and directly test.

Determination procedure:

1. Preheat spectrophotometer/ microplate reader for 30min, adjust the wavelength to 562 nm and set spectrophotometer counter to zero with distilled water.
2. Standard working solution: dilute 9μmol/mL NaNO2 standard solution with distilled water to 360, 180, 90, 45, 22.5, 11.25, 5.625 nmol/mL for use.
3. Add reagent as follows:

Reagent (μL)	Control tube	Test tube	Matrix-free tube	Blank tube	Standard

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	(Ac)	(At)	(Am)	(Ab)	tube (As)
Distilled water	-	-	8	8	8
Sample	8	8	-	-	-
Reagent I	112	112	112	112	112
Use a pipette to blow and mix thoroughly					
Reagent II	-	40	40	-	-
Standard	-	-	-	-	40
Distilled water	40	-	-	40	-
Mix well, accurately react in a 37 ° C water bath or constant temperature incubator for 3 min					
Reagent III	40	40	40	40	40

Mix and measure the absorbance at 562 nm in the micro glass cuvette/ 96 well plate, record it as Ac, At, Am, Ab and As. Calculate ΔA = (Am- Ab) - (At - Ac), ΔAs = As - Ab. Each test tube should be provided with one contrast tube. Standard curve and blank tube only need to be measured once or twice.

Calculation:

1. Standard curve drawing

Taking the concentration of each standard solution as the x-axis and its corresponding ΔA_S as the y-axis, draw a standard curve to get the standard equation y = kx + b, and bring ΔA_T into the equation to get x (μmol/mL).

2. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the oxidation of 1 nmol of Fe^{2+} per minutes every milligram tissue protein in the reaction system.

HP (U/mg prot) = x×V_2÷（Vs ×Cpr）÷T ×N= 1.667x÷Cpr×N

3. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the oxidation of 1 nmol of Fe^{2+} per min every gram tissue in the reaction system.

HP (U/g) = x×V_2÷（Vs×W÷Ve）÷T×N = 1.667x÷W×N

4. Liquid volume:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the oxidation of 1 nmol of Fe^{2+} every milliliter liquid in the reaction system per min.

HP (U/mL) = x×V_2÷Vs÷T ×N= 1.667x×N

V_2: the volume of reagent II added, 0.04mL.

Vs: sample volume added, 0.008mL;

Ve: volume used in the extraction solution, 1mL;

Cpr: sample protein concentration, mg/mL;

W: Fresh weight of sample, g;

T: React time, 3min.

N: Dilution factor.

Note:

1. When the determination of A is less than 0.1, it is recommended to dilute the crude enzyme solution with distilled water before performing the measurement, and multiply the dilution factor in the calculation formula.

Experimental Examples:

1. Take 0.1g of colon and add 1mL extract to homogenize and grind, take the supernatant and operate according to the measurement steps, and calculate ΔA = (Am- Ab) - (At - Ac) = （1.144-0.041）-（1.114-0.048）=0.037, bring standard curve line y=0.0031x-0.0007, x=11.710, calculate the enzyme based on the sample weight:

HP (U/g) =1.667×x÷W×N= 1.667×11.710÷0.1=195.161 U/g weight

2. Take rabbit serum and operate according to the measurement steps, calculate ΔA = (Am- Ab) - (At - Ac) =（0.577-0.048）-（1.114-0.048）=0.574, bring standard curve line y=0.0031x-0.0007, x=184.935, calculate the enzyme based on the liquid volume:

HP (U/mL) =1.667×x=1.667×184.935=308.287 U/mL

Related Products:

BC1300/BC1305 Ceruloplasmin(CP) Assay Kit
BC1310/BC1315 Total antioxidant capacity(T-AOC) Assay Kit
BC4430/BC4435 Uricase Activity Assay Kit
BC1360/BC1365 Uric acid（UA）Assay Kit
BC1320/BC1325 Hydroxyl Free Radical Scavenging Capacity Assay Kit

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