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Datasheet

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Total Amino Acids (T-AA) Colorimetric Assay

Cat# AS1002 96T

Store at 2-8℃ for 6 months

INTRODUCTION

This kit can be used to measure total amino acids (T-AA) content in serum, plasma, urine, animal and plant tissue samples.

BACKGROUND

Animal liver and kidney are the main organs of amino acid metabolism, so the change of the amino acid in urine can best reflect the physiological state of liver and kidney. In addition, amino acids can also react burns, typhoid, etc. Amino acid content in plant is of great significance to study changes of nitrogen metabolism, plants on the absorption of nitrogen, transport, assimilation and nutritional status under different conditions and different growth period.

PRINCIPLE of KIT

Copper ions can complex with various amino acids to produce blue-green complex compound, and the depth of color is proportional to the content of total amino acids at a specific wavelength. T-AA content can be calculated with the absorbance at 650 nm.

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution. The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring into the vial(s).

KIT COMPOENENTS & STORAGE

Item	Component	Specification	Storage
Reagent 1	Powder A	Powder × 1 vial	2-8℃ , 6 months
Reagent 2	Acid Reagent	0.8 mL × 1 vial	2-8℃ , 6 months
Reagent 3	Powder B	Powder × 1 vial	2-8℃ , 6 months
Reagent 4	Powder C	Powder × 1 vial	2-8℃ , 6 months
Reagent 5	Protein Precipitator	15 mL × 1 vial	2-8℃ , 6 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

MATERIALS PREPARED BY USERS

- Instruments

Test tube, Micropipettor, Vortex mixer, Microplate reader (640-660 nm, optimum wavelength: 650 nm), Centrifuge

- Reagents

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

➢ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

➢ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

THE KEY POINTS OF THE ASSAY

When preparing reagent 1 working solution, it is necessary to pay attention to whether the powder is completely dissolved.

REAGENT PREPARATION

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 1 working solution:
   Dissolve a vial of reagent 1 powder with 24 mL double distilled water, stir fully to form a blue turbid liquid, then add reagent 2 slowly and stir until the turbid liquid turns into light blue transparent liquid. Continue stirring for another 30 minutes, and the prepared solution can be stored at 2-8°C for 1 month.
3. Preparation of reagent 3 working solution:
   Dissolve a vial of reagent 3 powder with 12 mL double distilled water fully. The prepared solution can be store at 2-8°C for 1 month.
4. Preparation of 200 mmol/L standard:
   Dissolve a vial of reagent 4 powder with 5 mL double distilled water fully. The prepared standard solution can be stored at 2-8°C for 1 month.

SAMPLE PREPARATION

1. *Serum (plasma) and urine*: Detect the sample directly.
2. *Tissue sample*: Weigh the tissue accurately. Add normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) in a weight (g): volume (mL) ratio of 1: 9, homogenize mechanically in ice water bath to break tissue fully. Then centrifuge at 10000 g for 10 min at 4℃ and collect the supernatant for measurement. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

DILUTION OF SAMPLE

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (3.64-100 mmol/L).

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

Sample type	Dilution factor
Human serum	1
Human urine	1
Rat plasma	1
Porcine serum	1
10% Rat heart tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Mouse liver homogenate	1
10% Epipremnum aureum leaf tissue homogenate	1

Note: The diluent is reagent 5.

Assay protocol	
Ambient temperature	25-30℃
Optimum detection wavelength	650 nm

Instructions for the use of transferpettor

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

PLATE SET UP

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

OPERATING STEPS

- *The preparation of standard curve*

  Dilute 200 mmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 50, 60, 80, 100 mmol/L.

- *The measurement of samples*

1. *Standard tube*: Take 30 μL of standard with different concentrations to 1.5 mL EP tubes.

   Sample tube: Take 30 μL of sample to 1.5 mL EP tubes.
2. Add 120 μL of reagent 5 into each tube.
3. Mix fully with vortex mixer for 5 s and centrifuge at 3500 g for 10 min.
4. Take 100 μL of supernatant from each tube to 1.5 mL EP tubes.
5. Add 200 μL of reagent 1 working solution into each tube of step 4.
6. Mix fully with vortex mixer for 5 s.
7. Add 100 μL of reagent 3 working solution into each tube.
8. Mix fully with vortex mixer for 3 s, centrifuge at 3500 g for 10 min. Take 300 μL of supernatant to the microplate and measure the OD value of each well at

OPERATION TABLE

Standard tube Sample tube		
Standards with different concentrations (μL)	30	
Sample (μL)		30
Reagent 5 (μL)	120	120
Mix fully with vortex mixer for 5 s, centrifuge at 3500 g for 10 min and take 100 μL of supernatant for detection.		
Supernatant (μL)	100	100
Reagent 1 working solution (μL)	200	200
Mix fully with vortex mixer for 5 s.		
Reagent 3 working solution (μL)	100	100
Mix fully with vortex mixer for 3 s, centrifuge at 3500 g for 10 min. Take 300 μL of supernatant to the microplate and measure the OD value of each well at 650 nm with microplate reader.		

CALCULATION

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample.

The standard curve is: y= ax + b.

1. Serum (plasma) and other liquid sample

T-AA content (mmol/L) = (ΔA_{650} - b) ÷ a × f

2. Tissue sample

T-AA content (mmol/L) = (ΔA_{650} - b) ÷ a × f ÷ C_{pr}

Note:

y: $OD_{Standard}$ – OD_{Blank} (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of Standard. a: The slope of standard curve.

b: The intercept of standard curve.

f: Dilution factor of sample before tested.

ΔA_{650}: OD_{Sample} – OD_{Blank}.

C_{pr}: Protein concentration of sample, gprot/L.

NOTE

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.