Total Amino Acids Assay Kit

Catalog No.: abx298965

Size: 96 tests

Range: 3.64 mmol/L – 100 mmol/L

Sensitivity: 3.03 mmol/L

Storage: Store all components at 4°C for up to 6 months.

Application: For detection and quantification of Total Amino Acid concentration in serum, plasma, tissue, cell lysates, cell culture supernatants, urine, and other biological fluids

Introduction

Amino acids are organic compounds that contain an amino group and a carboxylic acid group. They are linked by amide bonds, forming polypeptides and proteins. Amino acids are essential for many biological processes, such as protein synthesis, neurotransmission and DNA synthesis. In animals, amino acids are mostly metabolized in the liver and kidney, and so the change of the amino acid in these tissues, and urine can reflect physiological changes in the body and organs. The role of amino acids in plant growth and nitrogen assimilation has become a major research target for the development of fertilizers.

Abbexa's Total Amino Acid Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Amino Acid concentration. The copper ion complex has an absorbance maxima at 650 nm. The intensity of the color is proportional to the Total Amino Acid content, which can then be calculated.

Kit components

1. 96-well microplate
2. Reagent A: 1 vial
3. Reagent B: 1 vial
4. Standard Powder: 1 vial
5. Acid Reagent: 0.8 ml
6. Protein Precipitator: 15 ml
7. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (650 nm)
2. Double distilled water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Centrifuge
7. Orbital shaker

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum, plasma, urine and other biological fluids:** Samples can be used directly.
- **Tissue Homogenates:** Weigh 0.02-1 g of tissue and wash with pre-chilled PBS. For each 1 g of tissue, add 9 ml of pre-chilled normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately. Dilute using the Protein Precipitator Solution as required. The protein concentration in the supernatant should be determined separately.

Samples should not contain detergents such as SDS, Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

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 Tissue Homogenate	1
10% *Epipremnum aureum* Tissue Homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Bring all reagents to room temperature before use**
- **Reagent A working solution:** Add 24 ml double distilled water to the Reagent A vial. Stir until the powder has fully dissolved, forming a blue cloudy solution. Add 0.7 ml of Acid Reagent slowly, and mix until the solution becomes transparent light blue. Continue mixing gently for 30 minutes with an orbital shaker. The working solution can then be stored at 4°C for up to 1 month.
- **Reagent B working solution:** Add 12 ml double distilled water to the Reagent B vial. Stir until the powder has fully dissolved. The working solution can then be stored at 4°C for up to 1 month.
- **200 mmol/L standard solution:** Dissolve a vial of Standard Powder with 5 ml double distilled water. The standard solution can then be stored at 4°C for up to 1 month. Ensure to mix the standard fully immediately before use.

B. Assay Procedure

1. Label 7 tubes, and prepare dilutions of the standard using double distilled water: 100, 80, 60, 50, 40, 20, 10 mmol/L. The distilled water itself serves as the 0 mmol/L (blank) standard.
2. Set sample and standard tubes. We recommend setting each sample and standard in duplicate.
3. Add 30 µl of the prepared standard to the standard tubes.
4. Add 30 µl of each sample to the sample tubes.
5. Add 120 µl of Protein Precipitator Solution to each standard and sample tube.
6. Vortex for 5 seconds, then centrifuge at 3500 x g for 10 minutes.
7. Take 100 µl of the supernatant from each tube and add to new centrifuge tubes.
8. Add 200 µl of Reagent A working solution to each tube from step 7. Vortex for 5 seconds to mix fully.
9. Add 100 µl of Reagent B working solution to each tube from step 8.
10. Vortex for 3 seconds, then centrifuge at 3500 x g for 10 minutes.
11. Set the standard and sample wells on the microplate, and record their positions.
12. Take 300 µl of the supernatant. Add the supernatant to the corresponding microplate well.
13. Measure the OD of each well with a microplate reader at 650 nm.

B. Calculation of Results

The standard curve should be plotted using the OD value and concentration of each standard. The standard curve is expected to be linear, and the concentration of each sample can be calculated by interpolation from the standard curve.

1. Serum, plasma, urine, and other biological fluids:

$$\text{Total Amino Acid Content (mmol/L)} = \frac{\Delta A_{650\,nm} - b}{a} \times f$$

2. Tissues samples:

$$\text{Total Amino Acid Content (mmol/L)} = \frac{\Delta A_{650\,nm} - b}{a} \times \frac{f}{C_{Protein}}$$

where:

$y = ax + b$	The linear equation of the standard curve
y	$(OD_{Standard} - OD_{Blank})$
x	The concentration of the standard
$\Delta A_{650\,nm}$	$(OD_{Sample} - OD_{Blank})$
$OD_{Standard}$	OD value of standard
OD_{Blank}	OD value of the blank standard (0 mmol/L)
OD_{Sample}	OD value of sample
a	The gradient of the standard curve (linear fit)
b	The intercept of the standard curve (linear fit)
f	The dilution factor of sample
$C_{Protein}$	Concentration of protein in sample (g/L)