Total Amino Acid

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1208

(Version 1.5D)

Detection and Quantification of Total Amino Acid Content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Amino acids are organic compounds that contain amine and carboxyl functional groups, along with a side chain specific to each amino acid. The key elements of an amino acid are carbon, hydrogen, oxygen, and nitrogen, although other elements are found in the side chains of certain amino acids. About 500 naturally occurring amino acids are known and can be classified in many ways. They can be classified according to the core structural functional groups' locations as alpha-, beta-, gamma- or delta-amino acids; other categories relate to polarity, pH level, and side chain group type. In the form of proteins, amino acid residues form the second-largest component of human muscles and other tissues. Beyond their role as residues in proteins, amino acids participate in a number of processes such as neurotransmitter transport and biosynthesis.

Total Amino Acid Colorimetric Microplate Assay Kit is designed to measure total amino acid directly in biological samples without any pretreatment. The intensity of the color, measured at 570nm, is directly proportional to the total amino acid concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent A Diluent	7.5 ml x 1	4 °C
Dye Reagent B	2.5 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 570 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. REAGENT PREPARATION

Dye Reagent: add 7.5 ml Dye Reagent A Diluent to Dye Reagent A dissolve before use, then add 2.5 ml Dye Reagent B, mix; store at 4 °C for 1 month after reconstitution.

Standard: Briefly centrifuge prior to opening. Add 1 ml Assay Buffer to dissolve before use, then add 0.1 ml into 0.9 ml Assay Buffer, the concentration will be 3 mmol/L, store at 4 °C for 1 month after reconstitution. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 3/1.5/0.75/0.375/0.187/0.0937/0.0468 mmol/L.

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Add 1 ml Assay Buffer for 0.1 ml serum or plasma; mix; centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Sample	100 µl	--	--
Standard	--	100 µl	--
Assay Buffer	--	--	100 µl
Dye Reagent	100 µl	100 µl	100 µl
Mix, keep at room temperature for 20 minutes, record absorbance measured at 570 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

** The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

VII. CALCULATION

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C = \frac{(OD_{Sample} - OD_{Blank}) - Intercept}{Slope} \times n \ (mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the protein concentration of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times C_{Protein} \times V_{Sample} \times V / (V + V_{Assay})} \ (\mu mol/mg)$$

2.2 According to the quantity of cells or bacteria

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times N \times (V_{Sample} / V_{Assay})} \ (\mu mol/10^4)$$

2.3 According to the weight of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay})} \ (\mu mol/g)$$

2.4 According to the volume of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times V / (V + V_{Assay})} \ (\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$V_{Standard}$: the volume of standard in assay procedure, mL

V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer, mL

$C_{Standard}$: the standard concentration, μmol/mL

$C_{Protein}$: the sample protein concentration, mg/mL

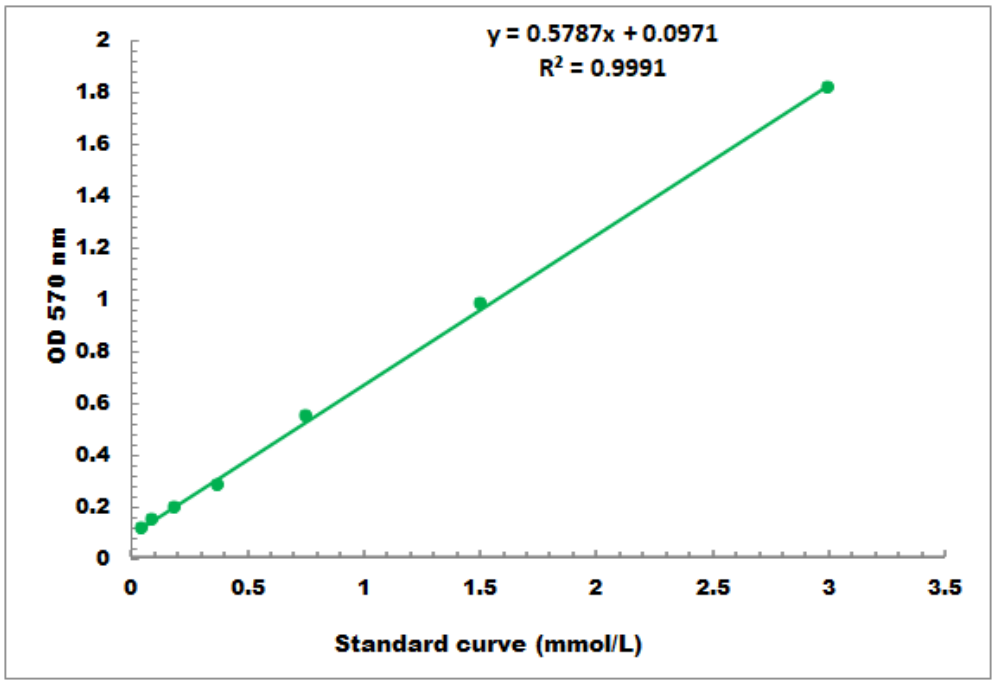
W: the weight of sample, g

V: the volume of sample in sample preparation, mL

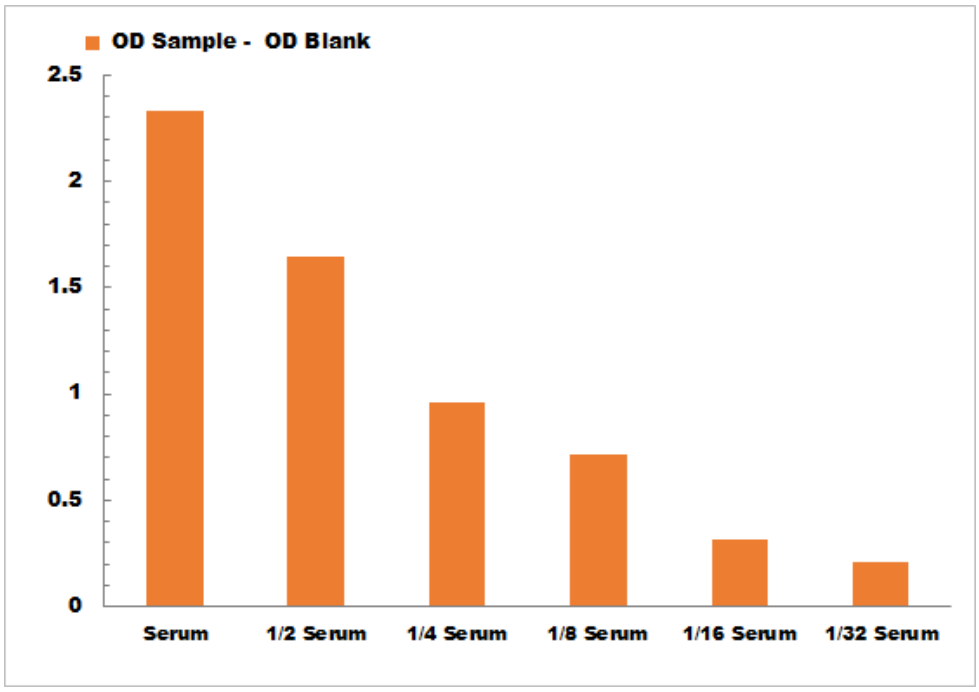
N: the quantity of cell or bacteria, $N \times 10^4$.

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.03 mmol/L - 3 mmol/L



Determination of total amino acid in serum