Ammonia/Ammonium Microplate Assay Kit User Manual

Catalog # FTA0164

(Version 1.2A)

Detection and Quantification of Ammonia/Ammonium content in Serum, Plasma, Urine, Saliva, Cell culture, Tissue extracts, Cell lysate, Other biological fluids Samples.

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

I. INTRODUCTION

Ammonia (NH3) or its ion form ammonium (NH4+) is an important source of nitrogen for living systems. It is synthesized through amino acid metabolism and is toxic when present at high concentrations. In the liver, ammonia is converted to urea through the urea cycle. Elevated levels of ammonia in the blood (hyperammonemia) have been found in liver dysfunction (cirrhosis), while hypoammonemia has been associated with defects in the urea cycle enzymes (e.g. ornithine transcarbamylase). Ammonia/Ammonium Microplate Assay Kit is designed to directly measure NH3 and NH4+ in a variety of samples. In the assay, ammonia reacts with hypochlorous acid, which is determined at 620nm, is directly proportional to the Ammonia/Ammonium concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 4	4 °C
Assay Buffer II	30 ml x 2	4 °C
Dye Reagent I	Powder x 1	4 °C
Dye Reagent II	3 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Dye Reagent I: add 7 ml distilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve, then add 5 µl into 995 µl distilled water, mix, the concentration will be 1000 µmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 620 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. 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 I for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and then add 0.5 ml Assay buffer II, mix.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer I, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and then add 0.5 ml Assay buffer II, mix.

3. For serum, plasma, urine and other biological fluids samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Sample	100 µl	--	--
Standard	--	100 µl	--
Distilled water	--	--	100 µl
Dye Reagent I	70 µl	70 µl	70 µl
Dye Reagent II	30 µl	30 µl	30 µl
Shake and mix, put it into the oven, 37 °C for 15 minutes. Then record absorbance measured at 620 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

VI. CALCULATION

1. According to the volume of sample

$$NH3\ (\mu mol/L) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 1000 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

2. According to the protein concentration of sample

$$NH3\ (\mu mol/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 1000 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

3. According to the weight of sample

$$NH3\ (\mu mol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay})$$

$$= 1500 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: the standard concentration, 1000 μmol/L;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

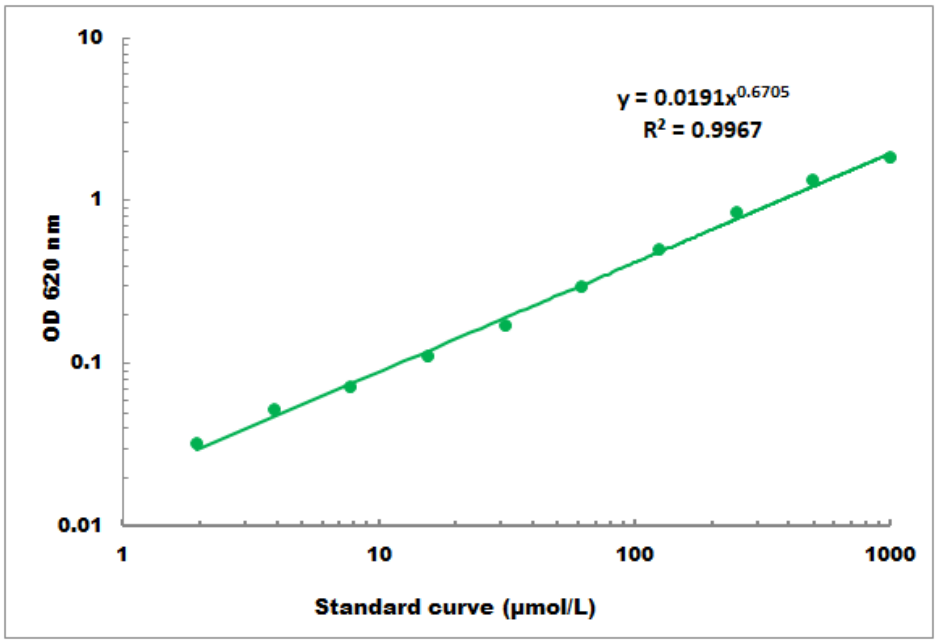
V_{Assay}: the volume of Assay buffer, 1.5 ml

$V_{Standard}$: the volume of standard, 100 μl;

V_{Sample}: the volume of sample, 100 μl.

VII. TYPICAL DATA

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



Detection Range: 1 μmol/L - 1000 μmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES