



# Ammonia/Ammonium Microplate Assay Kit User Manual

**Catalog # ASK1181**

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.**

Bioworld Technology, Inc. (USA)

Email: [info@bioworld.com](mailto:info@bioworld.com)

Web: [www.bioworld.com](http://www.bioworld.com)

Bioworld technology, co. Ltd. (China)

Email: [info@biogot.com](mailto:info@biogot.com)

Web: [www.biogot.com](http://www.biogot.com)



I. INTRODUCTION.....2

II. KIT COMPONENTS.....3

III. MATERIALS REQUIRED BUT NOT PROVIDED.....3

IV. SAMPLE PREPARATION.....4

V. ASSAY PROCEDURE.....5

VI. CALCULATION.....6

VII. TYPICAL DATA.....7



**I. INTRODUCTION**

Ammonia (NH<sub>3</sub>) or its ion form ammonium (NH<sub>4</sub><sup>+</sup>) 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 NH<sub>3</sub> and NH<sub>4</sub><sup>+</sup> 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
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  $5 \times 10^6$  cell or bacteria, sonicate (with power 20%, sonication 3s, interval 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:

<b>Reagent</b>	<b>Sample</b>	<b>Standard</b>	<b>Blank</b>
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.

**VI. CALCULATION**

1. According to the volume of sample

$$\begin{aligned} \text{NH}_3 (\mu\text{mol/L}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} \\ &= 1000 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

2. According to the protein concentration of sample

$$\begin{aligned} \text{NH}_3 (\mu\text{mol/mg}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times C_{\text{Protein}}) \\ &= 1000 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

3. According to the weight of sample

$$\begin{aligned} \text{NH}_3 (\mu\text{mol/g}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (V_{\text{Sample}} \times W / V_{\text{Assay}}) \\ &= 1500 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

$C_{\text{Standard}}$ : the standard concentration, 1000  $\mu\text{mol/L}$ ;

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

$W$ : the weight of sample, g;

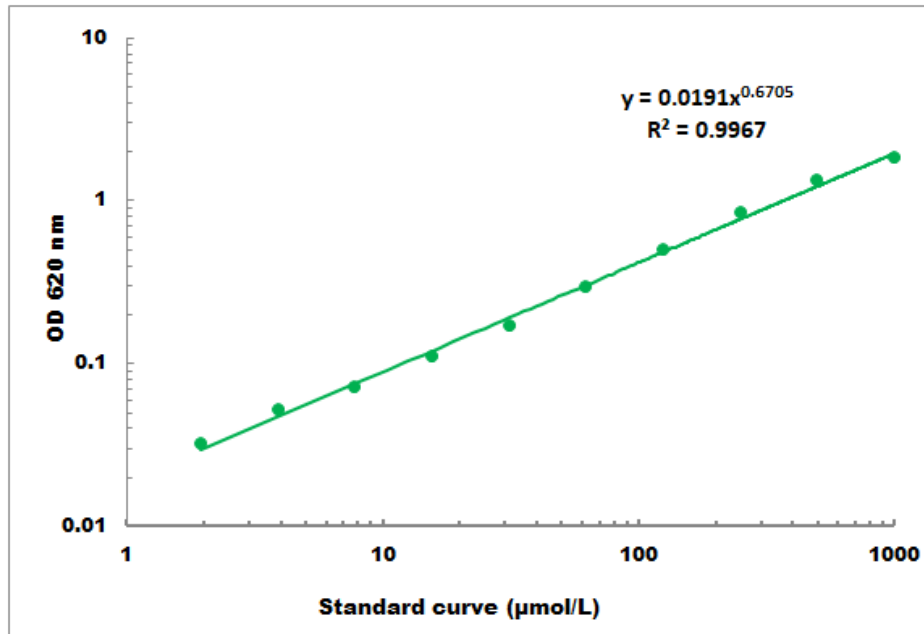
$V_{\text{Assay}}$ : the volume of Assay buffer, 1.5 ml

$V_{\text{Standard}}$ : the volume of standard, 100  $\mu\text{l}$ ;

$V_{\text{Sample}}$ : the volume of sample, 100  $\mu\text{l}$ .

**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