# Ammonia/Ammonium

# Colorimetric Microplate Assay Kit

# User Manual

**Catalog # CAK1181**

(Version 1.7D)

Detection and Quantification of Ammonia/Ammoniumcontentin Serum, Plasma, Urine, Saliva, Cell culture, Tissue extracts, Cell lysate, Other biological fluidsSamples.

**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/AmmoniumColorimetricMicroplate 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/Ammoniumconcentration in the sample.

## II.KIT COMPONENTS

| Component | Volume | Storage |
|---|---|---|
| 96-Well Microplate | 1 plate | |
| Assay BufferI | 30 mlx 4 | 4 °C |
| Assay BufferII | 30 mlx 2 | 4 °C |
| Dye Reagent I | Powder x 2 | 4 °C |
| Dye Reagent II | Powder x 2 | 4 °C |
| Dye Reagent II Diluent | 3 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 620 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

## IV. REAGENT PREPARATION

**Dye Reagent I:**Add 3.5 ml distilled waterinto 1 bottle Dye Reagent I to dissolve before use. Store at 4 °C and use within 24 hours.

**Dye Reagent II**: Briefly centrifuge prior to opening. Add 1.5 ml Dye Reagent II Diluentinto 1 vial Dye Reagent II,mix before use. Store at 4 °C and use within 24 hours.

**Standard:**Add 1 ml distilled water to dissolve, then add 5 µl into 995 µldistilled water, mix, the concentration will be 1000µmol/L.Store at 4°C for 1 month.Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 1000/500/250/125/62.5/31.2/15.6µmol/L.

## V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay Buffer I for $5\times10^6$ cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 10000g 4°C for 10minutes, 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 mlAssay BufferI, centrifuged at 10000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and then add 0.5 ml Assay Buffer II, mix.

3.For liquidsamples

For serum, plasma, urine and other contain protein samples, add 0.1 ml sample into 0.6mlAssay Buffer I, centrifuged at 10000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and then add 0.3 ml Assay Buffer II, mix.

For othersample does not contain proteins, detect directly.

## VI. 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 15minutes, then record absorbance measured at 620 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. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

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

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 volume of sample (contain proteins)

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

2.2 According to the volume of sample (does not contain proteins)

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

2.3 According to the cell and bacteria of sample

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

2.4 According to the weight of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of sample, g

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

$V_{Assay}$: the volume of Assay Buffer I and Assay Buffer II, mL

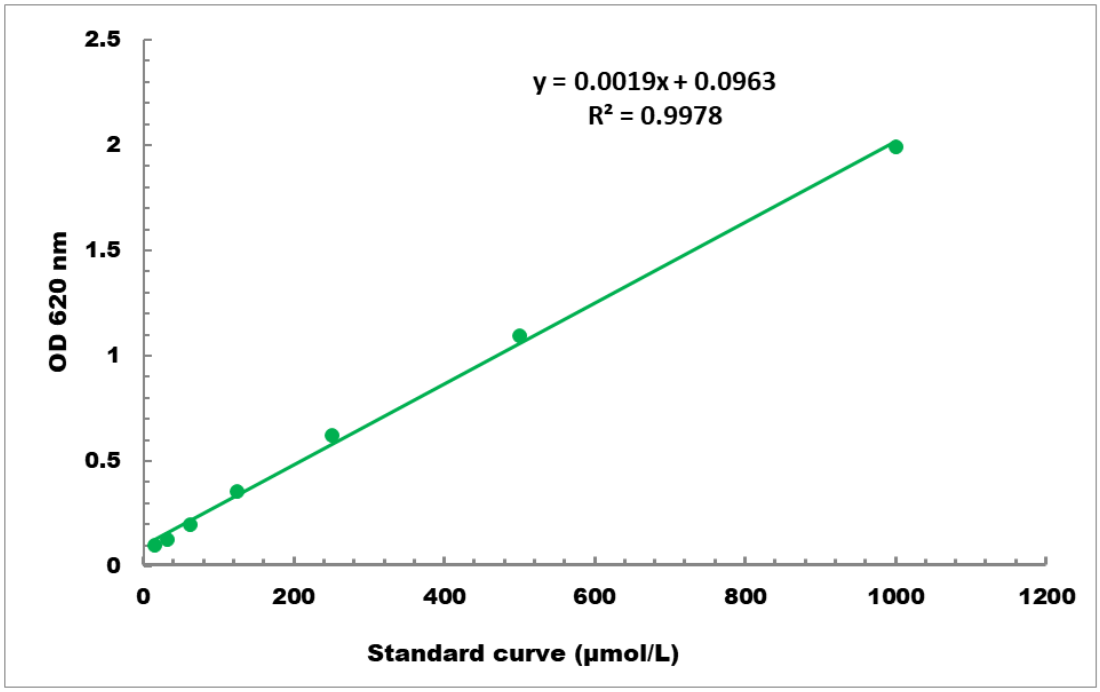
$V_{Standard}$: the volume of standard in assay procedure, mL

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

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

## VIII. TYPICAL DATA

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




Detection Range: 10μmol/L - 1000μmol/L