# BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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## Ammonia/Ammonium Assay Kit (Cat #: A-131)

**COMPONENTS:** AM Reagent 1: 10 ml (for 200 wells), store at 4ºC **(avoid light exposure)**
AM Reagent 2: 2 ml, store at 4ºC **(avoid light exposure)**
200 mM $NH_4Cl$: 0.1 ml, store at 4ºC

**PRODUCT DESCRIPTION:** The chromogenic Ammonia/Ammonium Assay Kit is based on an improved Berthelot method (Annals of Clinical Biochemistry 15, 270-275, 1978). Chemical reaction with ammonia/ammonium present in biological fluids (serum/plasma, saliva, urine), culture medium, or wastewater generates a product exhibiting an absorbance max at 660 nm. Assay sensitivity is typically 1 - 10 µM. Reagents are stable for at least 1 year if stored and handled properly.

## PROTOCOLS

**Ammonium chloride standards:** First dilute the 200 mM $NH_4Cl$ solution 1000x with $dH_2O$ to 200 µM, e.g., 1 µl 0.2 M solution + 999 µl $dH_2O$. Perform additional 1:1 serial dilution to obtain 100 µM, 50 µM, and 25 µM $NH_4Cl$ standards. Store diluted $NH_4Cl$ standards at 4°C.

### Preparation of reaction solution and control solution

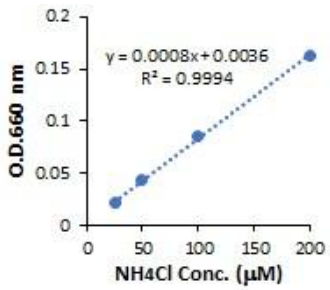
Each sample well requires 50 µl control solution and 50 µl reaction solution. Calculate the amount of each solution required for assay and prepare both solutions fresh prior to assay.

Reaction solution = 5 parts of AM Reagent 1 + 1 part of AM Reagent 2

Control solution = 5 parts of AM Reagent 1 + 1 part of $dH_2O$

## Assay setup

1. Use a plain (uncoated) 96-well plate for the assay. Pipet 10 µl $dH_2O$ to a well designated as blank well.
2. Pipet 10 µl of each $NH_4Cl$ standard (25, 50, 100, and 200 µM) to a set of wells.
3. Pipet 10 µl of each sample to duplicate wells (for sample control and sample reaction).
4. Add 50 µl reaction solution to the blank well, each $NH_4Cl$ standard well, and one set of sample wells (for reaction).
5. Add 50 µl control solution to another set of sample wells (for control). Gently agitate plate for 10 sec to mix contents. Incubate at 37°C for 30 min. *Do not use $CO_2$ incubator.*
6. Read absorbance (O.D.) at 660 nm using a plate reader.
7. Subtract blank well reading from each $NH_4Cl$ standard well reading. Use the subtracted $NH_4Cl$ readings to generate a trendline equation (see graph below).
8. Subtract sample control well reading from sample reaction well reading for each sample. Use the subtracted sample reading to calculate sample ammonia/ammonium concentration (x = concentration in µM; y = subtracted sample reading).




**ADDITIONAL INFORMATION**

- The assay kit contains salicylic acid, sodium nitroprusside, sodium hypochlorite, and sodium hydroxide. Please contact us or visit the product webpage for SDS information.