Ammonia Colorimetric Assay Kit

(Catalog #BN00632; 100 assays; Store kit at -20° C)

I. Introduction:

Ammonia is an important source of nitrogen for living systems. Nitrogen is required for the synthesis of amino acids, which are the building blocks of protein. Ammonia is a metabolic product which is created through amino acid deamination. It plays an important role in both normal and abnormal animal physiology such as acid/base balance. Assay Genie provides a rapid, simple, sensitive, and reliable assay suitable for research and high throughput assay of Ammonia or Ammonium. In the assay, Ammonia or ammonium is converted to a product that reacts with the GenieRed probe to generate color (OD 570 nm) which can be easily quantified by plate reader. The kit can detect 1 nmol (~20 µM) of ammonia or ammonium, much more sensitive than measuring NADPH based ammonia assay.

II. Kit Contents:

Components	100 assays	Cap Color	Part Number
Ammonia Assay Buffer	25 ml	WM	BN00632-1
GenieRed Probe in DMSO	200 µl	Red	BN00632-2
Enzyme Mix (lyophilized)	1 vial	Green	BN00632-3
Developer	1 vial	Orange	BN00632-4
Converting Enzyme (Lyophilized)	1 vial	Blue	BN00632-5
NH_4Cl Standard (10 mM)	100 µl	Yellow	BN00632-6

III. Storage and Handling:

Store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay. **All the solution in this kit should be kept capped when not in use to prevent absorption of ammonia from the air.**

IV. Reagent preparation:

GenieRed Probe: Warm to room temperature before use. Store at -20°C, protect from light and moisture.

Enzyme Mix, Developer and Converting Enzyme: Dissolve in 220 µl Assay Buffer separately. Aliquot to prevent multiple freeze/thaw cycle. Store at -20°C. Use within two months.

V. Ammonia Assay Protocol:

1. **Standard Curve Preparation:**
   Dilute the Ammonium Chloride standard solution to 1 mM by adding 10 µl of the 10 mM Ammonium Chloride Standard to 90 µl of ddH_2O, mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Ammonium Chloride Standard.

2. **Sample Preparations:**
   Tissues (20 mg) or cells ($2x10^6$) can be homogenized in 100 µl Assay Buffer, centrifuge at 13,000 x*g* for 10 minutes to remove insoluble material. Liquid sample can be tested directly. Add 2-50 µl sample to 96 well plate, bring the volume to 50 µl/well with Assay Buffer. For unknown samples, we suggest testing several different doses of samples to make sure the readings are within the standard curve range.

Note: Pyruvate in samples will interfere with the assay. If significant amount of pyruvate is suspected in your sample, set a Sample Control as in step 3. The pyruvate reading can be subtracted from sample readings.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix.

	Sample	Sample Control
Ammonia Assay Buffer	42 µl	44 µl
GenieRed Probe	2 µl	2 µl
Enzyme Mix	2 µl	2 µl
Developer	2 µl	2 µl
Converting Enzyme	2 µl	0 µl

Add 50 µl of the **Reaction Mix** to each well containing the NH_4Cl Standard and test samples. Add 50 µl Sample Control Mix to Sample Control. Mix well. Incubate the reaction for 60 min at 37°C, protect from light.

4. **Measurement:** Measure O.D. 570 nm in a micro plate reader.

5. **Calculation:** Correct background by subtracting the value derived from the 0 NH_4Cl from all readings (The background reading can be significant and must be subtracted from readings). Subtract the Sample Control readings from sample readings. Plot NH_4Cl standard Curve, NH_4Cl concentrations of the samples can then be calculated:

$$\mathbf{C = S_a/S_v \quad nmol/\mu l \text{ or } mM,}$$

where S_a is the sample amount (in nmol) from standard curve.
S_v is the sample volume (µl) added into the wells.
NH_4^+ Molecular Weight is 18.04 g/mol.

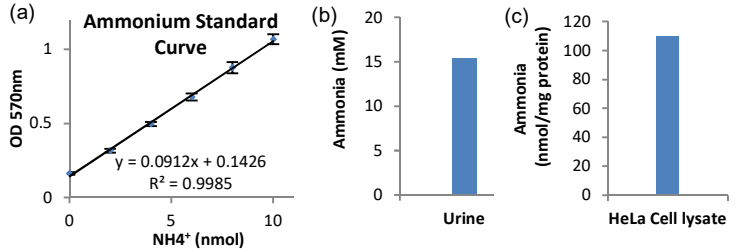

Figure. Ammonia Standard Curve (a). Ammonia concentration in human urine (25 µl, 100 times diluted) (b) and HeLa cell lysate (3 µl, 10.8 mg/ml) (c). Assay was performed according to the kit protocol.

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

AssayGenie

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		