BIOMEDICAL RESEARCH SERVICE CENTER
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Ammonia/Ammonium Assay Kit (Cat #: A-131)

COMPONENTS:

Ammonia Assay Solution (10x): 4 ml (100 wells), store at -80ºC
NADPH Solution (50x): 0.8 ml (brown vial), store at -80ºC
GLDH Control (50x): 0.4 ml, store at -80ºC
GLDH Reaction (50x): 0.4 ml, store at -80ºC
0.5 M NH_4Cl standard: 0.2 ml, store at 4ºC

PRODUCT DESCRIPTION: Amino acid catabolism represents the major source of ammonia production in the human body. Ammonia is toxic if the blood concentration goes above 40 μM. The activity of the urea cycle is designed to keep the peripheral blood concentration of ammonia below the toxic level. While ammonia toxicity in the newborns is almost always due to genetic defects in the urea cycle, ammonia toxicity in the adults usually results from liver damage due to alcohol, other poisons, or viral infection. The assay kit is based on the conversion of NADPH to $NADP^+$ in the presence of ammonia, α-ketoglutarate, and glutamate dehydrogenase. The decrease in optical density at 340 nm is proportional to the ammonia concentration in serum/plasma, urine, and culture medium. The assay requires a 0.1 ml Quartz cuvette for measuring kinetic changes in O.D.340 nm. Alternatively, a UV-transparent 96-well plate can be used. Reagents are stable for at least 1 year if stored and handled properly.

PROTOCOL

Ammonia Standard: First dilute the 0.5 M (500 mM) stock solution 1000 fold with dH_2O to 500 μM, i.e., 1 μl 0.5 M stock solution + 999 μl dH_2O. Perform additional serial dilutions to obtain 250 μM, 125 μM, and 62.5 μM standards. Store diluted standards at 4°C after use.

Preparation of assay solutions

Calculate the amount of control solution and reaction solution needed for each assay. Each sample and standard requires 0.2 ml control solution and 0.2 ml reaction solution freshly prepared prior to assay. Discard the solutions after each assay. An example for preparing 2 ml control solution and 2 ml reaction solution is given:

4 ml working solution = 3.6 ml dH_2O + 0.4 ml Ammonia Assay Solution + 80 μl NADPH Solution

2 ml control solution = 2 ml working solution + 40 μl GLDH Control

2 ml reaction solution = 2 ml working solution + 40 μl GLDH Reaction

Assay

1. Turn on spectrophotometer or plate reader. Set wavelength at 340 nm.
2. Pipet 20 μl of each standard and sample to one set of brown tubes (control tubes) and another set of brown tubes (reaction tubes). Pipet 20 μl of each standard and sample to duplicate wells if using a UV-transparent 96-well plate.
3. Add 0.2 ml control solution to each control tube/well followed by brief agitation. Add 0.2 ml reaction solution to each reaction tube/well followed by brief agitation. Incubate at 37°C for 20 min.
4. Read O.D.340 nm.
5. Subtract the reaction reading from the control reading for each standard and sample. Use the subtracted readings to generate an ammonium chloride standard plot.
6. Sample ammonia/ammonium concentration is derived from the trend line of the standard plot established at the same time (x= ammonia concentration in μM, y= $O.D._{340\ nm}$).