QuantiChrom™ Free Amino Nitrogen Assay Kit (DFAN-100)

Quantitative Colorimetric Free Amino Nitrogen Determination

DESCRIPTION

FREE AMINO NITROGEN (FAN) is the main source of nitrogen necessary for yeast growth and proper fermentation. Fermentation of beer and wine is processed by yeast, which synthesize proteins using available amino acids. When making beer and wine, free amino nitrogen is extracted from amino acids during the formation of the wort or must.

BioAssay Systems' Free Amino Nitrogen assay measures alpha amino acids, ammonia, and end group amino nitrogens. The ninhydrin based reaction is a superior method for determining only alpha amino acids and ammonia compared to the traditional Kjeldahl, which measures nitrogen from all sources. Only requiring low sample volumes, the stable ninhydrin reagent provides a simple and accurate method for determining Free Amino Nitrogen concentrations.

KEY FEATURES

Fast and sensitive. Linear detection range (5 µL sample): 0.2 to 10 mM for 10 min reaction.

Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated to process thousands of samples per day.

APPLICATIONS

Free Amino Nitrogen determination in foods and beverages (e.g. beer, wort, wine, must, etc.)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Reagent A: 18 mL **Standard:** 500 µL (20 mM Glycine)
Reagent B: 600 µL

Storage conditions. The kit is shipped at room temperature. Store all components at 4°C upon receiving. Shelf life: 12 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation

Beer, wort, wine and must samples should be diluted 10-fold in distilled water (n = 10).

All samples can be stored at –20 to 4°C for at least one month.

Reagent Preparation

Vortex reagent or warm in a bath if there are any particulates. Equilibrate all reagents to room temperature.

Procedure using 96-well plate

1. *Standards*. Prepare 200 µL 4 mM Premix by mixing 40 µL of the Standard (20 mM) and 160 µL distilled water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + H_2O	Glycine (mM)
1	100 µL + 0 µL	4
2	60 µL + 40 µL	2.4
3	30 µL + 70 µL	1.2
4	0 µL + 100 µL	0

2. Transfer 5 µL Standards into separate 1.5-mL centrifuge tubes.
3. Prepare enough Working Reagent (WR) for all assay wells by mixing, for each tube, 150 µL Reagent A and 5 µL Reagent B. Fresh reconstitution of the WR is recommended.
4. Add 100 µL WR to each sample tube. Close tube and vortex tube briefly to mix.
5. Incubate at 100°C for 10 min.
6. Allow tubes to cool to room temperature. Vortex and briefly centrifuge tubes (~1 min).
7. Transfer 100 µL from each reaction tube to separate wells of a microwell plate. Use a plate reader to read OD_{575nm}.

CALCULATION

Subtract blank value (water, #4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the Free Amino Nitrogen concentration of Sample as follows

$$\textbf{[Free Amino Nitrogen]} = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope}} \times \boldsymbol{n} \ \text{(mM)}$$

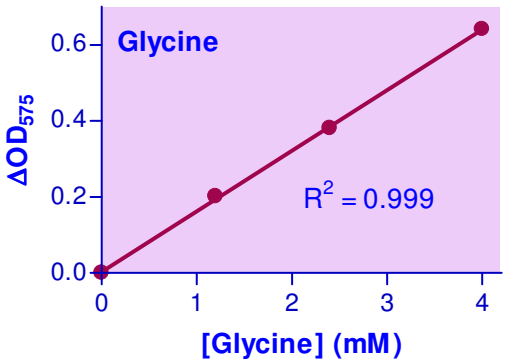
where OD_{SAMPLE}, OD_{BLANK} are optical density values of the Sample and H_2O Blank, respectively. *n* is the sample dilution factor. (*n* = 10 for beer, wort, wine and must samples.)

Note: if the calculated concentration is higher than 10 mM, dilute sample in water and repeat assay. Multiply the result by the dilution factor.

Unit conversion: 1 mM Glycine = 14 mg/L Nitrogen.

MATERIAL REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), incubator, centrifuge tubes and plate reader.



Glycine Standard Curve

LITERATURE

1. Thomas C.T., Ingledw WM (1990). Fuel Alcohol Production: Effects of Free Amino Nitrogen on Fermentation of Very-High-Gravity Wheat Mashes. *Applied and Environmental Microbiology*, Vol. 56, No. 7: 2046-2050.
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3. Pierce J.S. (1986). The Role of Nitrogen in Brewing. *J. Institute of Brewing*, Vol. 93: 378 – 381.