

PractiChrom™ Free Amino Nitrogen Assay Kit (PFAN-25)

Quantitative Colorimetric Free Amino Nitrogen Determination Using PICOEXPLORER™

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 synthesizes 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. Assay performed with portable PiCO Explorer device.

Cost efficient. No need for expensive plate readers.

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: 5 mL **Standard:** 500 µL (20 mM Glycine)

Reagent B: 150 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 ($n = 10$). Samples with particulates should be centrifuged before diluting the supernatant.

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 precipitates. Equilibrate all reagents to room temperature.

Procedure

- Standards.** Prepare 100 µL 4 mM Glycine Standard by mixing 20 µL of the Standard (20 mM) and 80 µL dH₂O.
- In separate PCR tubes, add 5 µL dH₂O (0 mM), 5 µL 4 mM Glycine Standard, and 5 µL of each sample.
- Prepare enough Working Reagent (WR) for water, standard, and sample tubes by mixing, for each tube, 150 µL Reagent A and 5 µL Reagent B. Fresh reconstitution of the WR is recommended.
- Add 150 µL WR to each 0 mM, 4 mM standard, and sample tube. Close tubes and briefly vortex or tap to mix.
- Incubate at 100°C for 10 min in water bath or heat block.
- Allow tubes to cool to room temperature. Vortex and briefly centrifuge tubes (~1 min).
- Please refer to the PICOEXPLORER™ User's Manual for detailed instructions for operating the device.
Download the PAS-110 application. Turn on Bluetooth.
Push the Power button on the device. Then, open the app and tap the Connection Setting button and connect the device.

Measuring a Standard Curve (See pg 17-19 in User's Manual)

Return to the main menu and tap the Standard Curve button. Set the following:

LED Output: 10%

Unit: mM

RBG Selection: G

Place the water tube into the device. Tap the known concentration input area (the box below 0.00), and click Measure. Remove the tube, place the 4 mM Glycine Standard in the device. Tap the box below 4.00 and click Measure. Click Graph to view the standard curve.

Measuring Sample Concentrations

Return to the main menu and tap the Measure button. Edit the LED output, Units, and RBG selection as done above for the standard curve. Record a Title for your data.

Place each Sample tube in the device and tap measure.

CALCULATION

The "concentration" will be displayed on the PICOEXPLORER™ for each Sample. To calculate the Free Amino Nitrogen concentration in the sample, multiply the Sample concentration by the dilution factor.

$$[\text{Free Amino Nitrogen}] = [\text{Sample}] \times n \quad (\text{mM})$$

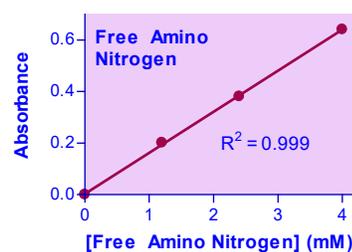
where [Sample] is the concentration of sample plus working reagent after incubation and n is the dilution factor.

Note: if the sample concentration says "Out of range" the sample is not within the linear range of the assay. If the color of the tube is pink like the 0 mM Glycine Standard tube, then the sample has low levels of Free Amino Nitrogen and should be diluted less than 10-fold. If the sample is very dark, dilute further in distilled water and repeat the assay. Multiply the results by the dilution factor.

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

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, PCR tubes (e.g. Watson 137-211c 0.2 mL; or Cat# PCR-50 from BioAssay Systems), Eppendorf tubes (e.g. Phenix Cat# MAX-715, or Cat # EPP-50 from BioAssay Systems), and PICOEXPLORER™ (Cat # PICO001).



Standard Curve in water measured with PICOEXPLORER

LITERATURE

- Thomas C.T., Ingledew WM (1990). Fuel Alcohol Production: Effects of Free Amino Nitrogen on Fermentation of Very-High-Gravity Wheat Mash. *Applied and Environmental Microbiology*, Vol. 56, No. 7: 2046-2050.
- Mosse J. (1990). Nitrogen to Protein Conversion Factor for Ten Cereals and Six Legumes or Oilseeds. A Reappraisal of Its Definition and Determination. Variation According to Species and to Seed Protein Content. *American Chemical Society*. 0021-8561/90/1438-0018.
- Pierce J.S. (1986). The Role of Nitrogen in Brewing. *J. Institute of Brewing*, Vol. 93: 378 – 381.