

Free Amino Nitrogen (FAN) Assay Kit (Colorimetric)

LS-K292-100 (100 Tests) • Store at 4°C



Introduction

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. This 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 μ L 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.)

Components

Component	K292-100
	100 Tests
Reagent A	18 mL
Reagent B	600 μ L
Standard (20 mM Glycine)	500 μ L

Materials Not Supplied

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.

Storage

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

FOR RESEARCH USE ONLY! Not for use in humans.

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

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Assay Procedure

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

- 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 ₂ O	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

- Transfer 5 μL Standards into separate 1.5 mL centrifuge tubes.
- 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.
- Add 150 μL WR to each sample tube. Close tube and vortex tube briefly to mix.
- Incubate at 100°C for 10 min.
- Allow tubes to cool to room temperature. Vortex and briefly centrifuge tubes (~ 1 min).
- Transfer 100 μL from each reaction tube to separate wells of a microwell plate. Use a plate reader to read $\text{OD}_{575\text{nm}}$.

Calculations

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

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

where $\text{OD}_{\text{SAMPLE}}$, OD_{BLANK} are optical density values of the Sample and H₂O 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.

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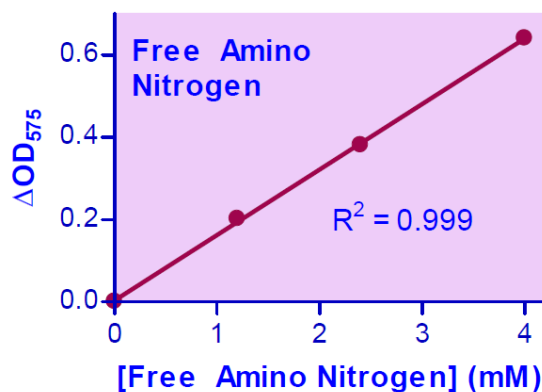
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Sample Data



Free Amino Nitrogen Standard Curve

Version: V.08.09.2018

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