

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 synthesise 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 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 with the traditional Kjeldahl method, which measures nitrogen from all sources. Requiring only low sample volumes, the stable ninhydrin reagent provides a simple and accurate method for determining free amino nitrogen concentrations.

Specifications

Detection Method	Colorimetric (OD 575 nm; ninhydrin-based)
Detection Range	0.2 - 10 mM (5 uL sample, 10 min reaction)
Sample Type	Foods and beverages such as beer, wort, wine and must
Species Reactivity	All
Assay Time	10 minutes (incubation at 100 degrees C)
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	Shipped at room temperature. Store all components at 4 degrees C upon receipt.
Shelf Life	6 months after receipt
Shipping	Room Temperature

Kit Components

Component	Quantity	Storage
Reagent A	18 mL	4 degrees C
Reagent B	600 uL	4 degrees C
Standard (20 mM Glycine)	500 uL	4 degrees C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0215	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Dilute beer, wort, wine and must samples 10-fold in distilled water (n = 10); all samples can be stored at -20 to 4 degrees C for at least one month.
2. Vortex the reagent or warm in a bath if there are any particulates, and equilibrate all reagents to room temperature.
3. Prepare 200 uL of 4 mM Premix by mixing 40 uL of the 20 mM Standard and 160 uL of distilled water, then dilute the standards in 1.5-mL centrifuge tubes as described in the standard table (4, 2.4, 1.2 and 0 mM).
4. Transfer 5 uL of each standard into separate 1.5-mL centrifuge tubes.

5. Prepare enough Working Reagent (WR) for all assay wells by mixing, for each tube, 150 uL of Reagent A and 5 uL of Reagent B; fresh reconstitution of the WR is recommended.
6. Add 100 uL of WR to each sample tube, close the tube and vortex briefly to mix.
7. Incubate at 100 degrees C for 10 minutes.
8. Allow the tubes to cool to room temperature, then vortex and briefly centrifuge the tubes (approximately 1 min).
9. Transfer 100 uL from each reaction tube to separate wells of a microwell plate and use a plate reader to read OD575nm.

Standard Dilution

No	Premix	+ H2O	Glycine (mM)
1	100 uL	0 uL	4
2	60 uL	40 uL	2.4
3	30 uL	70 uL	1.2
4	0 uL	100 uL	0

Calculation

Subtract the blank value (water, #4) from the standard values and plot the delta-OD against standard concentrations. Determine the slope and calculate the free amino nitrogen concentration of a sample as: [Free Amino Nitrogen] = [(OD_SAMPLE - OD_BLANK) / Slope] x n (mM), where OD_SAMPLE and OD_BLANK are the optical density values of the sample and H2O blank respectively, and n is the sample dilution factor (n = 10 for beer, wort, wine and must samples). If the calculated concentration is higher than 10 mM, dilute the sample in water and repeat the assay, then multiply the result by the dilution factor. Unit conversion: 1 mM Glycine = 14 mg/L nitrogen.

Notes

- 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 degrees C for at least one month.
- Vortex the reagent or warm in a bath if there are any particulates, and equilibrate all reagents to room temperature before use.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.

Assay Genie | sales@assaygenie.com | www.assaygenie.com

Technical specifications are based on manufacturer documentation and may be updated. Protocols shown are summaries - always follow the instructions supplied with your kit/lot.