Technical Bulletin

Free Amino Nitrogen Assay Kit

Catalog Number MAK449

Product 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.

The Free Amino Nitrogen Assay Kit measures alpha-amino acids, ammonia, and end group amino nitrogen. The ninhydrin based chromogenic reaction is a superior method for selectively determining alpha amino acids and ammonia compared to the traditional Kjeldahl digestion method, which measures nitrogen from all sources. The stable ninhydrin reagent provides a simple and accurate method for determining Free Amino Nitrogen concentrations. The linear range for the assay is 0.2 to 10 mM free amino nitrogen when using a 5 µL sample.

The kit is suitable for Free Amino Nitrogen determination in foods and beverages (e.g., beer, wort, wine, must, etc.).

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Reagent A 18 mL
  Catalog Number MAK449A
- Reagent B 600 µL
  Catalog Number MAK449B
- Standard (20 mM Glycine) 500 µL
  Catalog Number MAK449C

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes
- Microcentrifuge capable of RCF ≥ 14,000 × *g*
- Incubator capable of 100 °C
- Vortex mixer

Precautions and Disclaimer

For R&D Use Only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at 2-8 °C.

Preparation Instructions

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

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

- Beer, wort, wine, and must samples should be diluted 10-fold in purified water (DF = 10). All samples can be stored at –20 to 4 °C for at least one month. Transfer 5 µL of each Sample to a 1.5 mL microcentrifuge tube.

Standard Curve Preparation

1. Prepare a 4 mM Glycine Standard by mixing 40 µL of the 20 mM Glycine Standard with 160 µL of purified water.
2. Prepare Glycine Standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Glycine Standards

Well	4 mM Glycine Standard	Purified Water	Glycine mM
1	100 µL	-	4.0
2	60 µL	40 µL	2.4
3	30 µL	70 µL	1.2
4	-	100 µL	0

3. Mix well and transfer 5 µL of each Standard into separate 1.5 mL microcentrifuge tubes.

Working Reagent

Note: The Working Reagent should be prepared fresh for each assay run.

1. For each Standard and Sample well, mix 150 µL of Reagent A and 5 µL of Reagent B.
2. Add 150 µL of Working Reagent to each Standard and Sample microcentrifuge tube. Close reaction tubes and vortex briefly to mix.

Measurement

1. Incubate reaction tubes at 100 °C for 10 minutes.
2. Allow reaction tubes to cool to room temperature.
3. Vortex tubes to mix contents well, then centrifuge reaction tubes at 14,000 × *g* for ~1 minute.
4. Transfer 100 µL from each reaction tube to separate wells of a clear flat-bottom 96-well plate.
5. Read optical density (OD) at 575 nm.

Results

1. Subtract the OD_{Blank} (Standard #4) reading from the remaining Standard OD readings. Plot the corrected Standard OD readings against the Standard concentrations.
2. Determine the slope of the Standard curve using linear regression.
3. Calculate the Free Amino Nitrogen concentration of the sample:

Free Amino Nitrogen (as Glycine) (mM) =

$$\frac{(OD_{Sample} - OD_{Bla}}{Slope\ (mM^{-1})} \times DF$$

where

OD_{Sample} = OD value at 575 nm of Sample

OD_{Blank} = OD value at 575 nm of Blank (Standard #4)

Slope = Slope of the Standard Curve

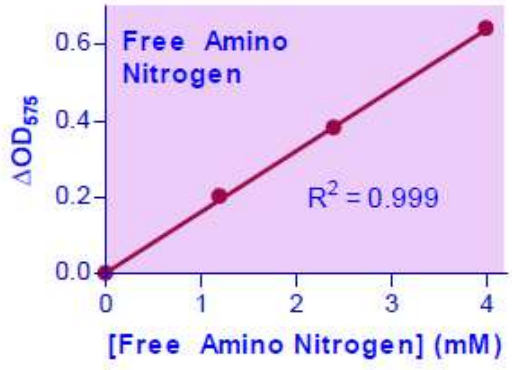
DF = Sample Dilution Factor (DF = 1 if sample is undiluted; DF = 10 for beer, wort, wine and must samples prepared as per the Sample Preparation instructions.)

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

Note: The Standard Curve is based on mM Glycine. To convert to concentration of nitrogen in the Sample, apply the unit conversion factor:

1 mM Glycine = 14 mg/L Nitrogen.

Figure 1.
Typical Free Amino Nitrogen Standard Curve



References

1. Thomas, C.T., and Ingledew, W.M., Fuel alcohol production: Effects of free amino nitrogen on fermentation of very-high-gravity wheat mashes. *Appl. Environ. Microbiol.*, **56(7)**, 2046-2050 (1990).
2. Mosse, J., 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. *J. Agric. Food Chem.*, **38(1)**, 18-24 (1990).
3. Pierce, J.S., The Role of Nitrogen in Brewing. *J. Instit. Brewing*, **93(5)**, 378-381 (1987).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Technical Service

Visit the tech service page on our web site at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.