



Free Amino Nitrogen Assay Kit (Colorimetric)

Free Amino Nitrogen Assay Kit (Colorimetric) is a detection kit for the quantification of Free Amino Nitrogen in food and beverages.

Catalog number: ARG82155

Package: 100 tests

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

In brewing and winemaking, free amino nitrogen (FAN) is a measure of the concentration of individual amino acids and small peptides (one to three units) which can be utilized by beer and wine yeast for cell growth and proliferation. Together with ammonia, FAN makes up the measurement of yeast assimilable nitrogen that can be measured prior to the start of fermentation.

The exact components of FAN will vary from composition of the wort or grape must. In wine, all 21 amino acids can be found in trace amounts with arginine, proline and glutamine being the most abundant. However, as *Saccharomyces cerevisiae*, the primary yeast for both beer and wine, can't utilize proline in the anaerobic conditions of ethanol fermentation it is not included in FAN (and subsequently YAN) calculations. [Provide by Wikipedia: Free amino nitrogen]

PRINCIPLE OF THE ASSAY

This Free Amino Nitrogen Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of free amino nitrogen (FAN) present in foods and beverages. 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.

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MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Reagent A	18 mL	4°C
Reagent B	600 µL	4°C
Standard (20 mM)	500 µL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 575 nm
- Centrifuge and centrifuge tube
- Incubator
- Clear flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

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SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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

Note:

- Samples can be stored at -20°C to 4°C for at least one month.

REAGENT PREPARATION

- **Working Reagent:** for each assay, mix 150 μL of Reagent A and 5 μL of Reagent B. Prepare immediately before assay.
- **Standards:** Prepare 200 μL of 4 mM Premix by mixing 40 μL of the Standard (20 mM) and 160 μL of distilled water. Dilute standard as follows.

Standard tube	Glycine (mM)	Distilled water (μL)	Standard Premix (μL)
S1	4	0	100
S2	2.4	40	60
S3	1.2	70	30
S4	0	100	0

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ASSAY PROCEDURE

Vortex reagent or warm in a bath if there are any particulates. Equilibrate all reagents to room temperature. Briefly centrifuge tubes before use.

1. Add **5 μ L** of **Standards** and **Sample** into separate **1.5 mL eppendorf**.
2. Add **100 μ L Working Reagent** to each sample eppendorf. Close tube and vortex tube briefly to mix.
3. Incubate for **10 minutes** at **100°C**.
4. Allow eppendorf to cool to **room temperature**. Vortex and briefly spin down (~1 minute)
5. Transfer **100 μ L** from each eppendorf to separate wells of a clear flat bottom 96 well microplate.
6. Read the absorbance at **O.D. 575 nm**.

CALCULATION OF RESULTS

1. Subtract blank value (distilled water, S4) from the standard values and plot the Δ OD against standard concentrations. Determine the slope and calculate the Free Amino Nitrogen concentration of Sample as follows:

$$\text{FAN (mM)} = [(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / \text{Slope}] \times n$$

Note:

- $\text{OD}_{\text{Sample}}$, OD_{Blank} : the O.D. 575 nm values of the sample and distilled water blank.
- 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 sample in

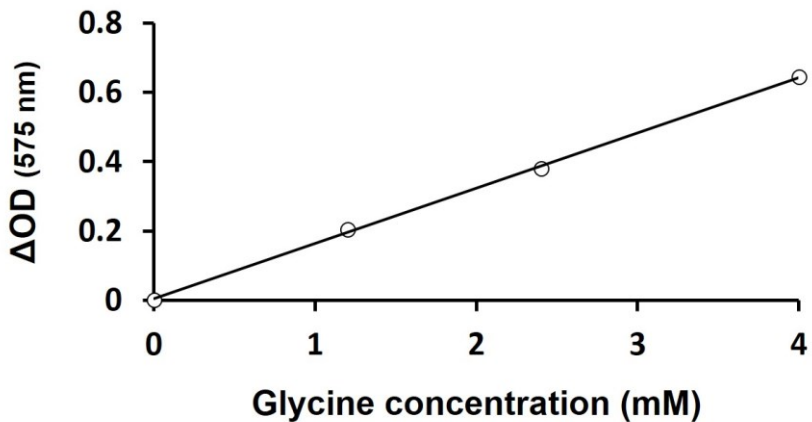
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distilled water and repeat assay. Multiply the result by the dilution factor.

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

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Free Amino Nitrogen Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.2 mM