

**Experimental Details Supplement to “Analysis of Protein and Total Usable Nitrogen in Beer Using a Microwell Ninhydrin Assay”, D. G. Abernathy, G. Spedding and Barry Starcher.
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MICROWELL PROTEIN ASSAY

Ninhydrin Stock Solution: Dissolve 8 g of ninhydrin in 300 mL of ethylene glycol and 100 mL of 4 N sodium acetate pH 5.5 buffer* (544 gm sodium acetate tetrahydrate + 400 ml glacial acetic acid in 1L water). Stable at room temperature for at least 6 months.

Stannous Chloride Solution: Dissolve 500 mg SnCl₂ in 5 mL ethylene glycol. Stable at room temperature for at least 6 months.

Ninhydrin Reagent/Working Solution: Prior to the assay add 25 uL of SnCl₂ solution to every 1 mL of ninhydrin stock solution and mix well.

Protein Standard (1ug/uL): Twenty milligrams of bovine serum albumen (Sigma Chem. Co, St. Louis MO) was hydrolyzed in 500 uL of 6 N HCl in a sealed microfuge tube at 100 °C for 18 h. The hydrolysate was microfuged and the supernatant brought to exactly 20 mL with distilled water.

Glycine Nitrogen Standard: Dissolve 107.2 mg glycine in distilled water and bring to exactly 100 mL.

Assay: 200ul beer + 200ul 12N HCl heat 20h at 100 °C. Cool, vortex and microfuge.

3 uL of beer hydrolysate is added in duplicate to Fisher 96 well microwell plates and 100 uL of working solution added to each sample.

The blank consisted of 100 uL working solution. A standard consisting of 5 uL of the protein standard is carried in duplicate with each assay. The plate is incubated at 104 °C for 10 min using a dry titerplate heater (Fisher). The absorption at 575 nm is measured with a Spectromax Plus (Molecular Devices). Net OD readings are converted to micrograms protein based on the OD readings from the 5 ug standard. For the calculations it must be kept in mind that the original beer sample was diluted in half with the addition of an equal volume of 12 N HCl.

Using this size sample and std. then %Protein
= $\frac{(\text{OD sample})}{(\text{OD standard})(3)}$

FAN ASSAY

Ninhydrin Color Reagent: Dissolve 4 g anhydrous Na₂HPO₄, 6 g KH₂PO₄, 0.5 g ninhydrin and 0.3 g fructose in a total of 100 mL distilled water. Store refrigerated upto 2 weeks in amber bottle.

Glycine Standard Stock: Dissolve 107.2 mg glycine in distilled water and bring to exactly 100 mL. Store at 0 - 4 °C.

Glycine Standard Solution: Dilute 1 mL glycine standard stock into 100 mL (final vol.) with distilled water. This standard contains 2 mg amino-nitrogen/L. Use freshly.

Dilution solution: Dissolve 2 g of potassium iodate (KIO₃) in 600 mL distilled water and add 400 mL 96% ethanol. Stable long-term at 4 °C.

Assay: 1 mL of beer is diluted to 50 mL with distilled water and 2 mL transferred to 16 X 150 mm test tubes. Ninhydrin color reagent (1 mL) is added and the tubes heated in a boiling water bath for 16 min. The tubes are transferred to a cold water bath and 5 mL of dilution reagent added, mixed and absorbance recorded at 575 nm against a blank containing 200 uL of water in place of the sample.

REDUCED VOLUME FAN ASSAY pH 5.5

Beer or grape juice (30 uL) or 20 uL (4 ug N) glycine standard is added to 200 ul of the pH 5.5 acetate buffered ninhydrin reagent *(see left column) and placed in a boiling water bath for 10 min. After 10 min the samples are removed and 2.8 mL cold water added, the tubes vortexed and the absorbance at 575 nm recorded against a blank containing 30 uL of water in place of the sample

MICROWELL FAN ASSAY

Add 2 uL (0.4ug N) of glycine standard or 2 uL of beer or grape juice to a 96 well microwell plate and add 100 uL of the pH 5.5 acetate buffered ninhydrin reagent. The plate is heated for 10 min at 104 °C. The absorbance is recorded at 575 nm on a microwell plate reader.

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