

# Quick, Reliable Results for Milk Protein Analysis by the Amido Black Technique using the Microlab 600

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# Introduction

The following colorimetric method enables quick determination of protein matter amounts in raw milk samples. The total protein level (TP) is a determining factor when establishing the commercial value of raw milk, and the proven reliability of this method is widely used in the milk processing industry.

## Principle

The dye-binding procedures used for milk protein testing employ different azosulfonic acid dyes : CI Acid Black 1 (or Amido Black), CI Acid Orange 12, and CI Acid Orange 10 (or Orange G). The mechanism of the reaction between the dye and the proteins is well understood. In acid buffer solution below the isoelectric point of the proteins, the positive charges of both the terminal amino groups, and the histidine, arginine and lysine residues, combine in a stoichiometric interaction with the negative sulfonic group of the dye to form an insoluble dye-protein complex. Lakin (1974) has shown that hydrophobic interactions occur between free and bound

anionic dyes. The differences between dyes, with respect to their sensitivity (change of absorbance per unit change of protein weight), are partly associated with their abilities to form hydrophobic interactions. Amido Black has the highest sensitivity followed by Acid Orange 12 and Acid Orange 10. injection, highlighting the durability found in all Hamilton Company HPLC columns.



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# Analysis

The measurement of the protein content is based upon the determination of the amount of unbound dye after removal of the dye-protein precipitate. Provided that the ratio of dye to protein remains within certain limits, a linear relationship exists between the protein content and the quantity of unbound dye.

Dye-binding method steps:

1. The milk sample (between 0.5 and 1.0 ml) is accurately diluted with the Microlab 600 with 20 mL of dye solution (Amido Black - pH of 2.2-2.4). After the dilution, the sample is thoroughly mixed to eliminate sedimentation.
2. Rapid partial equilibrium (ca. 5 s) between dye and proteins to react and form an insoluble complex.
3. A clear supernatant is obtained by filtration or centrifugation.
4. The absorbance of the supernatant is measured in a spectrophotometer, using a short path-length flow-through cuvette at a fixed wavelength, between 550 and 610 nm for Amido Black.

# Instrumentation

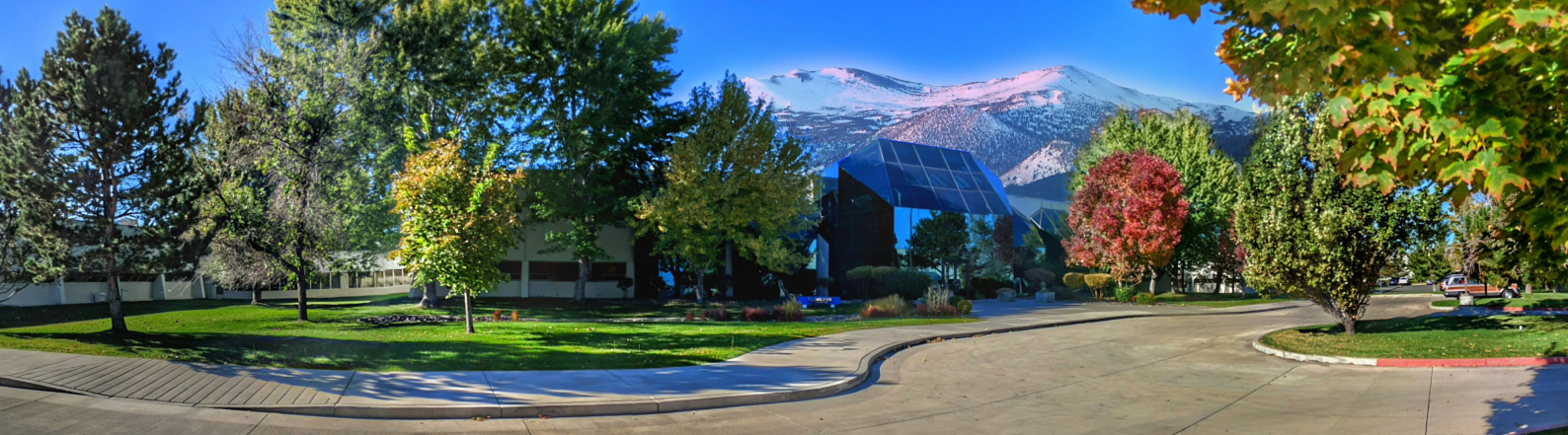
To perform a protein test by the Amido Black method, you will need:

1. Microlab 600 Dual Syringe Diluter, for sample preparation
2. Centrifuge
3. Spectrophotometer



For the above technique, accurate sample preparation is essential to achieve a low margin of error. The Microlab 600 positive displacement technology provides outstanding accuracy and precision as well as user independent handling to ensure excellent analysis results. The inert fluid path of the Microlab 600 is exclusively made of PTFE and borosilicate glass. The instrument is ideal for handling substances like Amido Black and can be easily cleaned after performing the sample preparation process.

Employing the Microlab 600 for diluting milk samples ensures highly accurate volume dispensing and excellent repeatability, while shortening sample preparation time.



## About Hamilton

Hamilton Company is a global manufacturer and supplier of world-class analytical components, medical instrumentation, temperature control systems, laboratory robotics and automated liquid handling equipment. Since its invention of the first lead-shielded syringe in 1947, Hamilton Company has set the standard for analytical fluid handling and measurement. This portfolio has since increased to include pipettes, polymeric HPLC columns, as well as process analytics sensors and equipment.

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