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MAX SIGNAL®

No-Mel Milk Total Protein Assay Manual

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GENERAL INFORMATION

Product Description

The *MaxSignal® No-Mel Milk Total Protein Assay* Kit is a colorimetric assay for the determination of protein in milk, powdered milk, cream, ice cream and chocolate drink.

MaxSignal® No-Mel Milk Total Protein Assay Kit uses a dye-binding assay to determine protein in milk and other dairy products. The kit enables international and government regulatory agencies, food manufacturers and processors, as well as quality assurance organizations, to determine protein levels in dairy products in response to customer concerns about food safety. The test is based on a proven AOAC-approved method for milk protein determination. The *MaxSignal® No-Mel Milk Total Protein Assay* Kit contains sufficient materials to test 42 dairy samples.

The assay detects the specific binding of milk proteins to dye molecules. This detection is fundamentally different than that of some other protein determination methods (such as the Kjeldahl method) which detect protein via nitrogen and are vulnerable to contamination of amine containing impurities such as melamine. The *MaxSignal® No-Mel Milk Total Protein Assay* Kit provides accurate, proven results even in the presence of high levels of melamine.

The unique features of the kit are:

- Rapid and simple method (modified version of AOAC method) less than 10 minutes.
- Minimal sample prep.
- Highly accurate and reproducible.
- Not influenced by the presence of melamine in samples ($\leq$ 2 mg/mL in milk).

The kit is designed to be used with a microplate reader or a UV/vis spectrophotometer (the preferred, most accurate method). The kit contains dye standards to construct a linear calibration curve and verify assay performance.

Procedure Overview

The *MaxSignal® No-Mel Milk Total Protein Assay* Kit measures the changes in soluble dye concentration caused by the presence of protein in dairy samples. The color change caused by consumption of the dye (measured by the absorbance at 490 nm) is related to the amount of protein present in the sample.

Kit Contents, Storage and Shelf Life

MaxSignal® No-Mel Milk Total Protein Assay Kit has the capacity for 96 determinations or testing of 42 samples in duplicate (using 12 wells for standards). Store the kit at room temperature. The shelf life is 6 months when the kit is properly stored.

Kit Contents	Amount	Storage
Microtiter Plate	1 x 96-well Plate (8 wells x 12 strips)	room temp
Calibration Standard: (protein equivalent concentration): *Std 1:* 0% protein *(white cap tube)* *Std 2:* 1% protein *(yellow cap tube)* *Std 3:* 2.5% Protein *(orange cap tube)* *Std 4:* 2.8% protein *(pink cap tube)* *Std 5:* 3.1% protein *(purple cap tube)* *Std 6:* 4% protein *(blue cap tube)*	 1.6 mL 1.6 mL 1.6 mL 1.6 mL 1.6 mL 1.6 mL	room temp
Dye Reagent	2 x 26 mL	room temp

Dairy Sample Types

- Homogenized Milk
- Low-fat Milk
- Milk Powder
- Cream
- Ice Cream
- Chocolate Drink

Required Materials/Equipment Not Provided With the Kit

- Spectrophotometer (digital instrument preferred) or microtiter plate reader (with 490 nm absorbance filter)
- 1 cm cuvettes (glass, quartz or polystyrene) or microplate
- Microcentrifuge tubes (polypropylene)
- Microcentrifuge

Warnings and Precautions

BIOO strongly recommends that you read the following warnings and precautions to ensure your full awareness of the techniques and other details you should pay close attention to when running the assays. Periodically, optimizations and revisions are made to the kit and manual. Therefore, it is important to follow the protocol coming with the kit. If you need further assistance, you may contact your local distributor or BIOO at techsupport@biooscientific.com.

- Do not use the kit past the expiration date.
- Do not intermix reagents from different kits or different lots.
- Try to maintain a laboratory temperature of 20°–25°C (68°–77°F).
- Avoid exposure of dye reagents to light.
- Make sure you are using only distilled or deionized water since water quality is very important.
- When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.

SAMPLE PREPARATION

Milk and Other Liquid Dairy Samples

1. Liquid samples can be tested directly without sample processing.
2. Be sure samples are properly stored. In general, samples should be refrigerated at 2-4°C for no more than 1-2 days.

Dry Milk

1. Reconstitute dry milk with appropriate volume of water according to the product label instruction, e.g. 9 -10 mL of water per gram of milk powder.
2. Mix thoroughly for at least 30 minutes to dissolve (preferably mix overnight).

   Note: *Dilution Factor = mL of water added per gram. For example, adding 10 mL of water to 1 gram of milk powder gives a dilution factor of 10.*

PROTEIN DETERMINATION TEST PROTOCOL

Set-up

Turn on instrument (spectrophotometer or plate reader), allow light source to warm up, and set absorbance wavelength to 490 nm. The accuracy of the results produced using a plate reader may be slightly less than those produced using a spectrophotometer due to shorter and slight variations in optical path length. The assay is most accurate using a spectrophotometer but a plate reader capable of measuring absorbance at 490 nm can also be used provided the samples are read in duplicate or (preferably) triplicate wells. When using a plate reader format, additional accuracy can also be achieved when a path length correction is performed. The accuracy of instruments other than spectrophotometers (i.e., plate readers) can be assessed from the resolution of the dye standards in the constructed calibration curve (see below).

Test Procedure

1. *Calibration curve*: Transfer exactly 1.25 mL of each Calibration Standard into 6 labeled clean microcentrifuge tubes. Add 70 µL of water to each tube.
2. *Dairy samples*: Dilute 70 µL of sample (such as milk) into exactly 1.25 mL dye reagent into a microcentrifuge tube. Also prepare duplicate samples of "blank" samples where 70 µL of water is added to the dye reagent instead of dairy sample.
3. Vortex mix the calibration standards and samples for 30 seconds.
4. Centrifuge at room temperature for 4 minutes at 11,000 rpm.
5. Transfer 0.7 mL of supernatant to a clean microcentrifuge tube. Avoid both the pellet at the bottom of the tube and lipids at the top surface of the sample (remove the supernatant from the middle). The sample is ready for the assay.
6. Transfer (in duplicate) the appropriate amounts of purified water and the sample to the cuvette or microplate (see table below).
7. Measure the absorbance at 490 nm.

Sample Holder	Volume Supernatant	Volume dH_2O	Total Volume
microplate	10 µL	290 µL	300 µL
1 mL cuvette	33 µL	967 µL	1000 µL
3 mL cuvette	100 µL	2900 µL	3000 µL

Calibration Curve Construction

Note: Although a calibration curve is *not* formally required to determine protein concentration using the *MaxSignal® No-Mel Milk Total Protein Assay* Kit (see "Data Analysis" section below), it is useful to confirm the accuracy of spectrophotometers and plate readers in the determination of milk protein concentration in individual labs.

For each calibration standard, plot the average absorbance at 490 nm as a function of protein concentration (percent protein). This plot should provide a tight linear curve with an R^2 value $\geq$ 0.99 for the linear fit.

DATA ANALYSIS

Calculation of Protein Concentration

$$\% \text{ protein} = 7.22 \times (1 - A_S/A_B)$$

where A_S is the average absorbance for the sample and A_B is the average absorbance of the "blank" samples where water was added to the dye instead of dairy sample.

A special program with Excel functionality, *MaxSignal® Total Protein Analysis Program in Excel*, is available upon request to evaluate the test results. Please contact your local distributor or techsupport@biooscientific.com for further information.

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