NY

AGRICULTURAL INDUSTRY STANDARD

OF THE PEOPLE'S REPUBLIC OF CHINA

ICS 67.120.01

B 45

NY/T 1663-2008

Determination of β-Lactoglobulin Content in Milk and Dairy Products -- SDS-PAGE Electrophoresis

乳与乳制品中 β-乳球蛋白的测定 聚丙烯酰胺凝胶电泳法

Issued on: July 14, 2008 **Implemented on: August 01, 2008**

Issued by: Ministry of Agriculture of PRC

Table of Contents

Determination of β-Lactoglobulin Content in Milk and Dairy Products -- SDS-PAGE Electrophoresis

1 Scope

This Standard specifies the determination of β-Lactoglobulin Content in Milk and Dairy Products by SDS-PAGE Electrophoresis.

This Standard is applicable to the determination of β-Lactoglobulin Content in Milk and Dairy Products (raw milk, milk powder, liquid milk, cheese, whey powder).

The detection limit of liquid samples in this standard is 24mg/100mL; while the detection limit of solid-liquid and solid samples is 240mg/100g.

2 Normative References

The provisions in following documents become the provisions of this Standard through reference in this Standard. For dated references, the subsequent amendments (excluding corrigendum) or revisions do not apply to this Standard, however, parties who reach an agreement based on this Standard are encouraged to study if the latest versions of these documents are applicable. For undated references, the latest edition of the referenced document applies.

GB/T 6682 Water for Laboratory Use - Specifications

3 Principle

After the sample is electrophoresed on SDS-PAGE gel, β-lactoglobulin is measured and analyzed with an optical densitometer to determine the content of β-lactoglobulin.

4 Reagents

Unless otherwise specified, only reagents confirmed to be analytically pure shall be used in the analysis; the experimental water shall comply with the provisions of Class-I water specified in GB/T 6682.

4.1 Glacial acetic acid (CH_3COOH)

4.20 Running gel buffer stock solution, 1.5 mol/L, pH 8.8

Accurately take 9.08g of tris(hydroxymethyl)aminomethane (4.9); dissolve it in 40 mL of water; adjust the pH to 8.8 by hydrochloric acid solution (4.17); use water to make the constant volume to 50mL; and store at 4°C.

4.21 Persulfate solution, 100g/L

Accurately take 0.1g of ammonium persulfate (4.4); add 1mL of water to dissolve; and prepare before use.

4.22 Dodecyl sulfonic acid sodium solution, 100g/L

Accurately take 5g of dodecyl sulfonic acid sodium (4.13); dissolve in water and make constant volume to 50mL; and store at room temperature.

4.23 N, N, N ', N'-Tetramethylethylenediamine solution with volume fraction of 10%

Take 0.10mL of N, N, N ', N'-Tetramethylethylenediamine (4.14); dilute with water and make constant volume to 1.00 mL.

4.24 Sample buffer, 0.08mol/L

Accurately take 4mg of bromophenol blue (4.15); dissolve it in 5.00mL water. Respectively, measure 1.60mL of stacking gel buffer stock solution (4.19), 4.00mL of dodecyl sulfonic acid sodium solution (4.22), 1.20mL of *β*-mercaptoethanol (4.10), 2.20mL of glycerin (4.7); all mixed, then dilute with water and make constant volume to 20mL; and stored at 4°C.

4.25 Electrode buffer, pH 8.3

Accurately take 3.0g of tris(hydroxymethyl)aminomethane (4.9) and 14.4g of glycine (4.8); add 10 mL of dodecyl sulfonic acid sodium solution (4.22); and adjust the pH to 8.3; make constant volume to 1000mL.

4.26 Coomassie brilliant blue staining solution, 2.5g/L

Take 0.25g of Coomassie brilliant blue R-250 (4.16) and 10g of ammonium sulfate (4.3); respectively, add 20mL of ethanol (4.6) and 10mL of phosphoric acid (4.2); dissolve and mix; and use water to make constant volume of 100mL.

4.27 De-staining solution

Respectively, take 75mL of glacial acetic acid (4.1), 50mL of methanol (4.5) and 875mL of water; and mix evenly.

4.28 *β*-lactoglobulin standard solution, 1.00 mg/mL

Accurately take 0.0100g of *β*-lactoglobulin standard substance (purity ≥90%); use the sample buffer (4.24) to make constant volume to 10mL; heat in a boiling water bath for 3min~5min; and store at -20°C below.

5 Apparatus

Commonly used laboratory instruments and the following items.

5.1 Balance with a sensitivity of 0.0001g.

5.2 Electrophoresis instrument.

5.3 Electrophoresis tank, 100mm×83mm.

5.4 Optical density scanner.

5.5 Micro syringe, 10μL.

5.6 Centrifuge, no less than 7000 r/min.

5.7 Magnetic-stirrer.

5.8 Centrifuge test tube with stopper.

6 Analysis Procedures

6.1 Specimen preparation

6.1.1 Liquid sample

Take 1mL of sample; sequentially add 1mL of water and 2mL of sample buffer (4.24); heat in boiling water bath for 3min~5min; stir by magnetic-stirrer (5.7) for 4h; centrifuge for 5min; remove fat; take supernatant and sub-package; store at -20°C for later-use.

6.1.2 Solid sample

Take 1g of sample, accurate to 0.1 mg; add appropriate amount of water to dissolve; make constant volume to 10mL; and operate according to 6.1.1.

6.2 Preparation of running gel

Prepare 20mL of 12% running gel according to Table 1; mix it and then add it to the gap between the long and short glass plates, which is about 60mm~70mm high. Slowly inject water about 5mm high along the long glass plate wall; and seal the water. After about 30min, the boundary between the gel and the water seal layer with different

6.8 De-staining

After staining the gel, first rinse its surface to remove the excessive stain; and then soak it into the de-staining solution (4.27) for de-staining. Change the de-staining solution until the gel background is colorless.

6.9 Analysis

Use the densitometer to measure and analyze the gel, and calculate the content of β-lactoglobulin according to the optical density value. For a typical map, see Appendix A.

7 Calculation of Results

The content of β-lactoglobulin in the specimen shall be calculated by the mass fraction, X; and the value is expressed in gram per 100 grams or gram per 100 milliliters (mg/100g or mg/100mL), and shall be calculated as per Formula (1):

$$X = \frac{OD_s}{OD_{std}} \times C_{std} \times \frac{V_s}{m} \times \frac{V_1}{V_2} \times f \times 100 \quad \cdots\cdots (1)$$

Where:

OD_s – optical density value of β-lactoglobulin in the specimen solution;

C_{std} – concentration of β-lactoglobulin in the standard solution, in mg/mL;

V_1 – constant volume of the sample, in mL;

OD_{std} – optical density value of β-lactoglobulin standard solution;

V_2 – sample loading volume of the specimen, in μL;

V_s – sample loading volume of β-lactoglobulin standard solution, in μL;

f – dilution factor;

m – specimen mass, in g.

The measurement result is expressed by the arithmetic mean value of the parallel determination; and three significant digits shall be retained.

NOT E: The correlation coefficient of the standard curve $r \geq 0.99$.

This is an excerpt of the PDF (Some pages are marked off intentionally)

------ The End ------