# QB

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## Cellulases

纤维素酶制剂

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# Foreword

**Clause 5.4 of this standard is mandatory, the rest is recommended.**

This standard makes reference to the “hygienic indicators” in the enzyme preparations for food industry of the Compendium of Food Additive Specification, Volume I, Joint FAO/WHO Expert Committee on Food Additive (JECFA). They are not equivalent.

Appendix A, Appendix B and Appendix C of this standard are normative appendixes, Appendix D is an informative appendix.

This standard was proposed by the China Light Industry Federation.

This standard shall be under the jurisdiction of the National Food Fermentation Standardization Center.

Drafting organizations of this standard: China Food Fermentation Industry Research Institute, Beijing Ningxiner Biotechnology Development Co., Ltd., Novozymes (China) Biotechnology Co., Ltd., Sino-French Joint Venture Tangshan Taiboer Biological Engineering Co., Ltd., Institute of Microbiology of Chinese Academy of Sciences.

The main drafters of this standard: Zhang Wei, Jiao Zhimin, Li Zhongxing, Zhai Wenjing, Zhao Li, Liu Jianjun, Cui Fumian, Tian Qijing.

This standard was the first release.

# Cellulases

## 1 Scope

This standard specifies the terms and definitions, requirements, test methods, inspection rules and markings, packaging, transportation and storage of cellulase.

This standard is applicable to the acidic (or neutral) cellulase which is prepared by refining and purifying microorganisms and their mutants represented by Trichoderma which has been subjected to liquid-submerged fermentation or solid culture. It is mainly used in food, textile, paper and other industries. Food grade cellulase can also be used as feed additives.

## 2 Normative references

The provisions in following documents become the provisions of this standard through reference in this standard. For the dated references, the subsequent amendments (excluding corrections) 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 191 Packaging - Pictorial marking for handling of goods

GB/T 4789.2 Microbiological examination of food hygiene-Detection of aerobic bacterial count

GB/T 4789.3 Microbiological examination of food hygiene - Detection of Coliform bacteria

GB/T 4789.4 Microbiological examination of food hygiene - Examination of salmonella

GB/T 5009.11 Determination of total arsenic and abio-arsenic in food

GB/T 5009.12 Determination of lead in foods

GB/T 8451 Method for limit test of heavy metals in food additives

QB/T 1803-1993 General methods of determination for industrial enzymes

QB/T 1804 General principles of inspection and mark, packing, transport,

storage for industrial enzymes

JJF 1070 Rules of metrological inspection for net content of prepackaged commodity with fixed content

Order No.43 of State Administration of Quality and Technical Supervision [1995]: Rules for supervision of quantitative packaged commodities

# 3 Terms, definitions, symbols, abbreviations

The following terms, definitions, symbols, and abbreviations apply to this standard.

**3.1**

**Cellulases**

The enzyme that, under the synergistic action of various enzyme components, can degrade the cellulose into cello-oligosaccharide, cellobiose and glucose.

**3.2**

**Filter paper activity (FPA)**

1 g of solid enzyme (or 1 mL of liquid enzyme), at (50 ± 0.1) °C and the specified pH (acid cellulase pH4.8, neutral cellulase pH6.0), is used to hydrolyze the filter paper substrate for 1 h, resulting in the amount of reducing sugar equivalent to 1 mg of glucose, which is one unit of enzyme activity, expressed in u/g (or u/mL).

**3.3**

**Sodium carboxymethylcellulose activity (CMCA)**

Reducing sugar method: 1 g of solid enzyme (or 1 mL of liquid enzyme), at (50 ± 0.1) °C and the specified pH (acid cellulase pH4.8, neutral cellulase pH6.0), is used to hydrolyze the sodium carboxymethylcellulose substrate for 1 h, resulting in the amount of reducing sugar equivalent to 1 mg of glucose, which is one unit of enzyme activity, expressed in u/g (or u/mL) and abbreviated as CMCA-DNS.

Viscosity method: 1 g of solid enzyme (or 1 mL of liquid enzyme), at (40 ± 0.1) °C and the specified pH (acid cellulase pH6.0, neutral cellulase pH7.5), is used to hydrolyze the sodium carboxymethylcellulose substrate, to reduce the viscosity of the substrate, thus obtaining the relative enzyme activity of the cellulase corresponding to the standard, abbreviated as CMCA-VIS.

It is determined in accordance with the method of Appendix C.

**6.4 pH**

It is determined in accordance with clause 9 of QB/T 1803-1993.

**6.5 Heavy metals**

It is determined in accordance with GB/T 8451.

**6.6 Lead**

It is determined in accordance with GB/T 5009.12.

**6.7 Arsenic**

It is determined in accordance with GB/T 5009.11.

**6.8 Total number of colonies**

It is determined in accordance with GB/T 4789.2.

**6.9 Coliforms**

It is determined in accordance with GB/T 4789.3.

**6.10 Salmonella**

It is determined in accordance with GB/T 4789.4.

# 7 Inspection rules and marking, packaging, transportation, storage

**7.1** Package, storage and transportation pictorial signs shall be implemented in accordance with GB/T 191.

**7.2** For food grade cellulases, they shall be marked with the words “food grade” on the label (or quality certificate).

**7.3** Except for the clauses as mentioned above, it is carried out in accordance with QB/T 1804.

and 21.89 g of disodium hydrogen phosphate dihydrate, DISSOLVE it in 10 L of deionized water. ADJUST the pH of the solution to (6.0 ± 0.05), PREPARE for use. The solution can be stored for one month at room temperature.

**A.2.4** Glucose standard stock solution (10 mg/mL)

WEIGH 1 g of anhydrous glucose which has been dried to constant weight at (103 ± 2) °C, accurate to 0.1 mg, USE water to dissolve it, to make its volume reach to 100 mL.

**A.2.5** Glucose standard use solution

Respectively TAKE 0.00, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50 mL of glucose standard stock solutions in a 10 mL volumetric flask, USE water to dilute it to 10 mL, CAP it, SHAKE it uniformly to prepare for use.

The above series of concentrations shall be adjusted as needed.

**A.2.6** Quick qualitative filter paper (Hangzhou Xinhua No.1 filter paper), Φ15 cm (each batch of filter paper is corrected by standard enzyme before use).

**A.3 Instruments**

In addition to ordinary laboratory instruments, there shall also be:

**A.3.1** Spectrophotometer

**A.3.2** Acidity meter: accuracy ± 0.01 pH

**A.3.3** Constant temperature water bath: (50 ± 0.1) °C

**A.3.4** Analytical balance: sensitivity amount 0.1 mg

**A.3.5** Magnetic stirrer

**A.3.6** Stopwatch or time clock

**A.3.7** Boiling water bath (it can be composed of 800 W electric furnace and high-beast beaker, enamel measuring cup or other container)

**A.3.8** Stoppered test tube: 25 mL

**A.4 Analytical procedures**

**A.4.1** Drawing standard curve

In accordance with the amount specified in Table A.1, respectively TAKE the glucose standard use solution (A.2.5), buffer solution (A.2.2 or A.2.3) and DNS reagent (A.2.1) in each tube (3 samples made in parallel for each tube), MIX it

respectively, (place it vertically along 1 cm direction).

- Respectively in four test tubes, accurately ADD 1.50 mL of the buffer solution of corresponding pH (A.2.2 or A.2.3).

- Accurately ADD 0.50 mL of the diluted enzyme solution to be determined (A.4.2.1) to the three sample tubes (not added into the blank tube), respectively, so that the filter paper is immersed in solution in the tube, CAP it.

- Place four test tubes in a (50 ± 0.1) °C water bath at the same time, accurately MAKE timekeeping, LET it react for 60 min, TAKE it out.

- Immediately and accurately ADD 3.0 mL of DNS reagent (A.2.1) to each tube. Then, accurately ADD 0.50 mL of the diluted enzyme solution (A.4.2.1) to the blank tube, SHAKE it uniformly. PLACE the four tubes in a boiling water bath at the same time, HEAT it for 10 min, TAKE it out, quickly COOL it to room temperature, ADD water to make its volume reach to 25 mL, SHAKE it uniformly.

- USE the blank tube to adjust the instrument's zero point. At the wavelength 540 nm of the spectrophotometer, USE the 10 mm cuvette to respectively determine the absorbance of the sample solution in the three parallel tubes, TAKE the average value. USE the average absorbance to check the standard curve or otherwise the linear regression equation to calculate the content of the reducing sugar.

**A.5 Calculation of results**

The FPA enzyme activity is calculated in accordance with formula (A.1).

$$X_1 = A \times 1/0.5 \times n \quad \cdots\cdots (A.1)$$

Where:

$X_1$ - Filter paper activity (FPA) of the sample, u/g (or u/mL):

A - The amount of reducing sugar found (or calculated) from the standard curve based on absorbance, mg;

1/0.5 - Converted to 1 mL of enzyme solution:

n - Enzyme sample's dilution factor.

**A.6 Allowable difference**

The absolute difference between the two determination results of the same

# Appendix B

## (Normative)

### Determination method of sodium carboxymethylcellulose (reducing sugar method) activity (CMCA-DNS)

### B.1 Principle

The cellulase hydrolyzes the cellulose substrate (sodium carboxymethylcellulose) at a certain temperature and pH, to release the reducing sugars. Under alkaline and boiling conditions, 3,5-dinitrosalicylic acid (DNS reagent) reacts with reducing sugars, its color depth is proportional to the content of reducing sugar (by glucose). The amount of reducing sugar produced is obtained by measuring the absorbance at 540 nm, the CMCA-DNS enzyme activity of the cellulase is calculated, which is used to represent the enzyme activity of the cellulase.

### B.2 Reagents and solutions

Unless otherwise stated, only analytically pure reagents and distilled or deionized water or water of comparable purity are used in the analysis.

**B.2.1** Sodium carboxymethylcellulose (CMC-Na)

Chemically pure (Shanghai Guanghua Chemical Reagent Factory) at 25 °C, 2% water solution, viscosity 800 mPa•s ~ 1200 mPa•s. (Each batch of sodium carboxymethylcellulose is calibrated with standard enzymes prior to use).

**B.2.2** Sodium citrate buffer solution, 0.05 mol/L pH4.8 (for acid cellulase)

WEIGH 4.83 g of sodium citrate monohydrate, DISSOLVE it in about 750 mL of water, ADD 7.94 g of trisodium citrate whilst stirring it, USE water to dilute it to 1000 mL, ADJUST the pH of the solution to (4.8 ± 0.05), PREPARE for use.

Note: It may also use the pH4.8 acetic acid buffer solution: WEIGH 8.16 g of sodium acetate trihydrate, DISSOLVE it in about 750 mL of water, ADD 2.31 mL of acetic acid, USE water to make its volume reach to 1000 mL. ADJUST the pH of the solution to (4.8 ± 0.05), PREPARE for use.

**B.2.3** Phosphate buffer solution, 0.1 mol/L pH6.0 (for neutral cellulase)

Respectively WEIGH 121.0 g of sodium dihydrogen phosphate monohydrate and 21.89 g of disodium hydrogen phosphate dihydrate, DISSOLVE it in 10 L of deionized water. ADJUST the pH of the solution to (6.0 ± 0.05), PREPARE for use. The solution can be stored for one month at room temperature.

# Appendix C

## (Normative)

### Determination method of sodium carboxymethylcellulose (viscosity method) activity (CMCA-VIS)

### C.1 Principle

Cellulase degrades sodium carboxymethylcellulose at a certain temperature and pH, the viscosity of the substrate decreases accordingly, the decrease in viscosity is proportional to the activity of endo-cellulase. Through comparison between the value as measured by viscometer and the standard enzyme sample of known enzyme activity, the relative enzyme activity (mainly endo-nuclease activity) of the cellulase to be determined is converted.

### C.2 Reagents and solutions

**C.2.1** Sodium citrate buffer solution, 0.1 mol/L pH6.0 (for acid cellulase)

Respectively WEIGH 121.0 g of sodium dihydrogen phosphate monohydrate and 21.89 g of disodium hydrogen phosphate dihydrate, DISSOLVE it in 10 L of deionized water. ADJUST the pH of the solution to (6.0 ± 0.05), PREPARE for use. The solution can be stored for one month at room temperature.

**C.2.2** Phosphate buffer solution, 0.1 mol/L pH7.5 (for neutral cellulase)

WEIGH 22.49 g of sodium dihydrogen phosphate monohydrate, 148.98 g of disodium hydrogen phosphate dihydrate, 10.0 g of PEG 6000 (polyethylene glycol), DISSOLVE it in 10 mL of deionized water. ADJUST the pH of the solution to (7.5 ± 0.05), PREPARE for use. The solution can be stored for one month at room temperature.

**C.2.3** Sodium carboxymethylcellulose (CMC-Na)

BLANOSE 7LF (Hercules Incorporated) at 25 °C, the degree of substitution of 65% ~ 90%, 2% aqueous solution, viscosity of 20 mPa•s ~ 50 mPa•s.

**C.2.4** CMC-Na solution

WEIGH 35 g of CMC-Na, accurate to 1 mg, slowly ADD about 700 mL of the corresponding buffer solution (C.2.1 or C.2.2), HEAT it to 80 °C ~ 90 °C, while magnetically stirring and heating it, until CMC-Na is completely dissolved. After cooling, USE the corresponding buffer solution (C.2.1 or C.2.2) to make its volume reach to 950 mL, USE 2 mol/L hydrochloric acid or sodium hydroxide to adjust the pH of the solution to (6.0 ± 0.05) (acid cellulase) or (7.5 ± 0.05)

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