

JIS

JAPANESE INDUSTRIAL STANDARD

Amylase for industrial use

JIS K 7001^{—1990}

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In the event of any doubt arising,
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Amylase for industrial use

K 7001-1990

1. Scope

This Japanese Industrial Standard specifies the amylase for industrial use (hereafter, referred to as amylase.).

Remarks 1. Applicable standards in this Standard are shown in Attached Table 1.

2. The units and numerical values given in { } in this Standard are based on the traditional units and are appended for informative reference.

2. Common matters

General matters common to the chemical analysis shall be as given in JIS K 0050.

3. Definitions

For the main terms used in this Standard the definitions in JIS K 0211 and JIS K 3600 apply, and the rest of the terms shall be as follows:

- (1) liquefying power Liquefying power means activity which acts on starch paste, hydrolyzes its glucoside chain from the inside to be converted to lower molecular weight and thereby, decreases the viscosity of the starch paste.
- (2) saccharification power Saccharification power means activity which acts on starch and its decomposed product or the like, hydrolyzes its glucoside chain, and generates reducing force.
- (3) sterilization Sterilization means activity which kills or removes all micro-organisms in the subject substance (however, viruses are excepted).

4. Classification

Amylase shall be classified as given in Table 1.

Table 1. Classification of amylase

Classification			Action	Principal use
Bacteria liquefied type amylase EC 3.2.1.1	For food	High heat resistance	α -1, 4-glucoside chain of starch or the like are decomposed from the inside, which is converted to lower molecular weight to be liquefied.	Starch liquefaction (manufacture and brewing of glucose)
		Middle-heat resistance		
	For non food	High heat resistance		Desizing of a fabric
		Middle heat resistance		
Glucoamylase EC 3.2.1.3			α glucoside chain of starch or the like is decomposed successively from its unreduc- ed terminal by a glucose unit.	Manufacture of glucose
β -amylase EC 3.2.1.2		Refined product	α -1, 4 glucoside chain of starch or the like is decom- posed successively from its unreduced terminal by a maltose unit.	Manufacture of maltose
		General product		
Pullulanase EC 3.2.1.41			α -1, 6 glucoside bond which is a branch point of a mole- cular of starch, pullulan, etc. is decomposed.	Manufacture of glucose and maltose.

Remarks 1. The EC numbers as given in Table 1 are specified by the enzyme committee of International Biological Organization.

2. There are a powder product and a liquid product in each product.

5. Quality

The quality of amylase shall be as given in Table 2.

Table 2. Quality of amylase

Items		Classification							Test method	
		Bacteria liquefied amylase				Gluco-amylase	β- amylase			Pullula-nase
		For food		For non food			Refined product	General product		
		High heat resistance	Middle heat resistance	High heat resistance	Middle heat resistance					
Activity	Liquefying power ⁽¹⁾ JLU/g or JLU/ml	1 000 min.		300 min.		—	—	—	—	7.1
	Saccharification power ⁽²⁾ JGU/g or JGU/ml	—		—		5 000 min.	—	—	—	7.2
	Saccharification power ⁽³⁾ JMU/g or JMU/ml	—		—		—	5 000 min.	1 000 min.	—	7.3
	Saccharification power ⁽⁴⁾ JPU/g or JPU/ml	—		—		—	—	—	100 min.	7.4
Heavy metal (as Pb) wt ppm		40 max.		—		40 max.	40 max.	40 max.	40 max.	7.6
Arsenic (as As ₂ O ₃) wt ppm		3 max.		—		3 max.	3 max.	3 max.	3 max.	7.7.1 or 7.7.2
Number of general bacteria piece/g or piece/ml		1×10 ⁴ max.		—		1×10 ⁴ max.	1×10 ⁴ max.	—	1×10 ⁴ max.	7.8

Notes ⁽¹⁾ When amylase acts on paste of 91 g/l in final concentration and 6.0 in pH equivalent to 1 g of starch at 65°C for 15 min, an enzyme quantity required for decreasing kinematic viscosity of this paste to 250 × 10⁻⁶ m²/s {250 cSt} (measured at 65°C) is taken as 1 liquefying power unit (JLU).

⁽²⁾ 1 % (in reaction liquid) soluble starch is made to act on an enzyme at 4.5 in pH and 40°C for 10 min. An enzyme quantity required for generating reduction force equivalent to 1 μmol glucose for 1 min, under this condition is taken as 1 saccharification power unit (JGU).

⁽³⁾ 1 % (in reaction liquid) soluble starch is made to action an enzyme at 5.5 in pH and 40°C for 10 min. An enzyme quantity required for generating reduction force equivalent to 1 μmol glucose for 1 min under this condition is taken as 1 saccharification power unit (JMU).

⁽⁴⁾ 0.2 % (in reaction liquid) pullulan solution is made to react on an enzyme at 5.0 in pH and 40°C for 30 min. An enzyme quantity necessary for generating reduction power equivalent to 1 μmol glucose for 1 min under this condition is taken as 1 saccharification power unit (JPU).

6. Sampling method

The sampling method shall be as follows.

6.1 Liquid product Divide into each manufacturing batch, sample at random a number of containers equivalent to 10 % or over of the number of containers in said batch from each batch, take an equal amount of liquid from each container into another container by using a liquid sampler as given in Fig. 1, and immediately hermetically seal. After sufficiently mixing, take approx. 50 ml therefrom, and use it as a sample.

6.2 Powdery product Sample a packed container in the same purport as in 6.1, take an equal quantity from each packaged container by using a powdery sampler as given in Fig. 2, and mix the whole thereof. Thereafter, reduce by a quartering method, sample approx. 50 g, and use it as a sample.

Fig. 1. Liquid sampler

Unit: mm

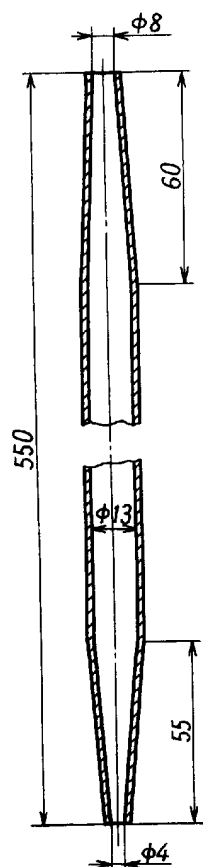
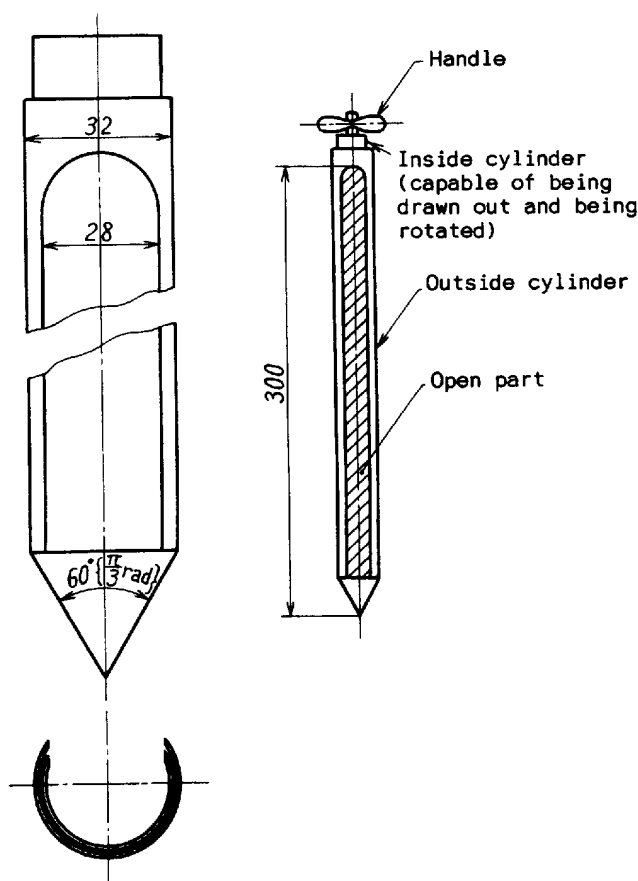


Fig. 2. Powdery sampler

Unit: mm



7. Test method

The test method shall be as follows.

7.1 Liquefying power Make enzyme act on white potato starch as a substrate, and measure the viscosity of starch which decreases as the starch is wholly being converted to a low molecule.

7.1.1 Reagents and apparatus The reagents and apparatus shall be as follows.

(1) Reagents

- (a) Boric acid buffer solution (7.0 in pH) Boric acid buffer solution (7.0 in pH) is prepared by dissolving 26.35 g of boric acid specified in JIS K 8863 and 7.15 g of sodium tetraborate + hydrate specified in JIS K 8866 in water to make 1000 ml.
- (b) Liquid for dilution [8.0 in pH⁽⁵⁾] Liquid for dilution (8.0 in pH) is prepared by dissolving 0.344 g of calcium sulfate dihydrate specified in JIS K 8963 in water and by adding 20 ml of boric acid buffer solution (7.0 in pH), 0.5 ml of polyoxyethylene(10) octylphenyl ether solution⁽⁶⁾, and water to make 1000 ml.
- (c) Acetic acid (1 mol/l) Acetic acid (1 mol/l) is prepared by adding water to 60 g of acetic acid specified in JIS K 8853 to make 1000 ml.
- (d) Sodium acetate solution (1 mol/l) Sodium acetate solution (1 mol/l) is prepared by dissolving 136 g of sodium acetate trihydrate specified in JIS K 8371 in water to make 1000 ml.
- (e) Acetic acid buffer solution (6.0 in pH) Acetic acid buffer solution (6.0 in pH) is prepared by adding acetic acid (1 mol/l) to sodium acetate solution (1 mol/l) to regulate its pH at 6.0 (approx. 95:5 in mixing ratio).
- (f) Substrate liquid Substrate liquid is prepared by weighing out accurately white potato starch (white potato starch in Pharmacopoeia Japonica of approx. 17 % in loss on drying) corresponding to 10.00 g as an anhydride and by adding 10 ml of acetic acid buffer solution (6.0 in pH) and water to make accurately 100 ml. It shall be prepared for each test.
- (g) Viscosity comparing liquid Silicone⁽⁷⁾ (dimethylpolysiloxane).

Notes (5) When boric acid buffer solution (7.0 in pH) is diluted 50 times with water, pH becomes 8.0.

(6) Add water to 10 g of polyoxyethylene(10) octylphenyl ether (for instance, Triton X-100), dissolve by warming, and make 100 ml.

(7) Silicone oil of $500 \pm 25 \times 10^{-6} \text{ m}^2/\text{s}$ { $500 \pm 25 \text{ cSt}$ } in kinematic viscosity and 0.60 ± 0.02 viscosity temperature coefficient at 25°C. Its kinematic viscosity at 65°C is $250 \pm 20 \times 10^{-6} \text{ m}^2/\text{s}$ { $250 \pm 20 \text{ cSt}$ }.

(2) Apparatus

- (a) Test tube A test tube of 18 mm in barrel outside diameter and 180 mm in height.
- (b) Thermostatic water bath A thermostatic water bath capable of regulating at $65 \pm 0.5^\circ\text{C}$.

(c) Boiling water bath

- (d) Viscosity comparing liquid sealing tube A viscosity comparing liquid sealing tube is prepared by enclosing 11 ml (11.34 g) of viscosity comparing liquid of (1) (g) in the same material, same form test tube as that to be used in a test. Two pieces thereof shall be prepared.

7.1.2 Preparation of sample solution Add a suitable quantity of liquid for dilution (8.0 in pH) to a sample enzyme, dissolve, and take it as a sample solution ⁽⁸⁾. If necessary, perform warming extraction and filtration.

Note ⁽⁸⁾ The enzyme quantity in sample solution is almost inversely proportional to time required for decreasing the kinematic viscosity of reaction liquid to $250 \times 10^{-6} \text{ m}^2/\text{s}$ {250 cSt} thereby. Therefore, where an unknown sample is tested, test operation is performed by using firstly an optional sample solution to obtain time required for the kinematic viscosity of reaction liquid to reach $250 \times 10^{-6} \text{ m}^2/\text{s}$ {250 cSt}. The enzyme quantity in 1 ml of said sample solution is estimated therefrom, and prepare a series of concentration sample solution corresponding to the enzyme of concentrations at approx. 5 % intervals centering 1 JLU/ml base thereon.

7.1.3 Operation method The operation method shall be as follows.

- (1) Quickly measure 10.0 ml of basic liquid violently shaking so that starch is uniformly dispersed, and put it into a test tube.
- (2) Add 1 ml of sample solution to 10 ml of substrate liquid in a test tube by using a transfer pipet.
- (3) Heat the test tube in boiling water bath violently shaking the test tube so that starch is uniformly dispersed⁽⁹⁾. When the starch is impasted, immediately transfer to a thermostatic water bath at $65 \pm 0.5^\circ\text{C}$.
- (4) After 15 min has accurately elapsed since it is transferred to the thermostatic water bath, put the test tube containing reaction liquid between two viscosity comparison liquid sealing test tubes preliminarily dipped onto the same thermostatic water bath, hold three test tubes collecting them, take them out from the thermostatic water bath, quickly incline the mouth part downward at approx. $\pi/4$ rad (45°) from a horizontal line hanging their bottom parts on an edge of the water bath, and observe the fluid state of liquid in the test tube⁽¹⁰⁾.
- (5) The enzyme quantity in 1 ml of sample solution when the sample solution flows from the reaction liquid at the same speed as that of viscosity comparing liquid is taken as 1 JLU⁽¹¹⁾.

Notes ⁽⁹⁾ Stopper a test tube with a silicone rubber stopper. After dispersing uniformly starch by shaking violently, quickly take the stopper out, and immediately start heating. If a boiling water bath filled with hot water is to be used in heating, the operation becomes easy to be performed. When an unreacted pasty lump is observed on the bottom of the test tube after liquefying reacting liquid, retest.

Notes (¹⁰) When observing operation is successively repeated where high heat resistance amylase is tested, a phenomenon wherein reaction liquid flows extremely quicker for the second time than that for the first time and thereafter, the flow is stabilized, is seen. Then, an observing operation is performed totally two times 10 s and 5 s before specified reaction time (15 min), and the test tube is returned to a thermostatic water bath each time. The third observing operation is performed when the reaction time reaches just 15 min, and the judging is performed. For middle heat resistant amylase, there is no quick variation in flowing of the reaction liquid like that for high heat resistant amylase.

(¹¹) Where among a series of sample solution enzyme concentrations of which are different, kinematic viscosity of reaction liquid 15 min later by one sample solution is higher (lower) than $250 \times 10^{-6} \text{ m}^2/\text{s}$ {250 cSt} and that of the sample solution of the following interval concentration is lower (higher), the sample concentration equivalent to 1 JLU/ml is taken as the average of concentrations of both sample solutions.

7.1.4 Calculation The liquefying power of a sample enzyme shall be calculated from the following formula.

$$a=b$$

where, a : liquefying power (JLU/g or JLU/ml)

b : dilution multiple

7.2 Saccharification power (glucoamylase) Make an enzyme react on soluble starch as a substrate, and measure reduction power, to be generated by a Fehling-Lehman-Shoul modification method.

7.2.1 Reagents and apparatus The reagents and apparatus shall be as follows.

(1) Reagents

- (a) Acetic acid buffer solution (4.5 in pH) Acetic acid buffer solution (4.5 in pH) is prepared by adding sodium acetate solution (1 mol/l) specified in 7.1.1 (1) (d) to acetic acid (1 mol/l) specified in 7.1.1 (1) (c) to regulate pH at 4.5 (approx. 57.5:42.5 in mixing ratio) and by diluting this solution 10 times.
- (b) Substrate liquid Suspend 10 g of soluble starch anhydride specified in JIS K 8659 with a small quantity of water, gradually cast it into 400 ml of boiling water, and take care so that boiling is not interrupted. After boiling for 5 min, cool in running water. Thereafter, add water to make 500 ml, and take it as substrate liquid. Prepare it for each test.
- (c) Fehling liquid A Fehling liquid A is prepared by dissolving 69.3 g of copper (II) sulfate pentahydrate specified in JIS K 8983 in water to make 1000 ml.

- (d) Fehling liquid B Fehling liquid B is prepared by dissolving 346 g of sodium potassium tartrate tetrahydrate specified in JIS K 8536 and 103 g of sodium hydroxide specified in JIS K 8576 in water to make 1000 ml.
- (e) Potassium iodide solution Potassium iodide solution is prepared by dissolving 300 g of potassium iodide specified in JIS K 8913 in water to make 1000 ml.
- (f) Sulfuric acid (250 g/l) Sulfuric acid (250 g/l) is prepared by carefully adding 250 g of sulfuric acid specified in JIS K 8951 to approx. 250 ml of water and by adding water after cooling to make 1000 ml.
- (g) Starch solution Mix 1 g of soluble starch with a small quantity of water, gradually add into 50 ml of boiling water so that boiling is not stopped, and keep for approx. 2 min. Thereafter, cool, and make 100 ml by adding water to obtain the starch solution.
- (h) 0.05 mol/l sodium thiosulfate solution 0.05 mol/l sodium thiosulfate solution is prepared by dissolving 50 g of sodium thiosulfate pentahydrate specified in JIS K 8637 and 0.2 g of sodium carbonate (anhydride) specified in JIS K 8625 in the water which is freshly boiled and cooled for 2 min to make 1000 ml. In order to preserve from decay, 2 ml of 3-methyl-1-butanol (isoamyl alcohol) specified in JIS K 8051 may be added. Though it is reserved as stock solution, dilute 4 times with the water which is once boiled and cooled at least two days before use, and reserve in a brown bottle. Perform standardization every day for one week after preparation and thereafter, once every three days.

Standardization Dry potassium iodide specified in JIS K 8005 at 120 to 140°C for 2 h. After standing to cool in a silica gel desiccator, weigh out approx. 0.36 g to the nearest 0.1 mg, dissolve in water, put into a 250 ml volumetric flask, and add water to the mark. Take 25 ml thereof into a 300 ml Erlenmeyer flask with ground stopper, add 2 g of potassium iodide and 5 ml of hydrochloric acid (2 + 1), immediately tightly stopper, quietly mix, and allow to stand in a dark place for approximately 5 min. After adding approximately 100 ml of water thereto, titrate free iodine with this sodium thiosulfate solution, and when the yellow of the solution becomes light, add 1 ml of starch solution as an indicator. Titrate until the blue color of generated starch iodide disappears. Perform correction by separately performing a blank test under the same condition, and calculate the factor (f) from the following formula.

$$f = c \times \frac{d}{100} \times \frac{25}{250} \times \frac{1}{x \times 0.0017833}$$

- where,
- c : weighed out quantity of potassium iodide (g)
 - d : content of potassium iodide (%)
 - x : 0.05 mol/l sodium thiosulfate solution required for titration (corrected value) (ml)
 - 0.0017833 : quantity of potassium iodide equivalent to 1 ml of 0.05 mol/l sodium thiosulfate solution (g)

(2) Apparatus

- (a) Test tube A flat bottom test tube (30 mm in barrel inside diameter and 120 mm in height) with aluminium cap.
- (b) Thermostatic water bath A thermostatic water bath capable of regulating at $40 \pm 0.5^\circ\text{C}$.
- (c) Boiling water bath
- (d) Buret A buret of 0.1 ml in minimum scale.

7.2.2 Preparation of sample solution Dissolve a sample enzyme in an appropriate quantity of water and prepare the sample solution to become 1 to 5 JGU/ml degree.

7.2.3 Operation method The operation method shall be as follows.

- (1) Take 3 ml of substrate liquid and 2 ml of acetic acid buffer solution into a test tube, and keep it in a thermostatic bath at $40 \pm 0.5^\circ\text{C}$ for 10 to 15 min.
- (2) Add 1.0 ml of the sample solution warmed at $40 \pm 0.5^\circ\text{C}$ thereto, sufficiently mix by shaking, immediately put into the thermostatic bath at the same temperature, and allow to stand accurately for 10 min (start reaction).
- (3) Add 2.0 ml of Fehling liquid B, and sufficiently mix by shaking (stop reaction).
- (4) Add 2.0 ml of Fehling liquid A. After lightly mixing by shaking, apply a cap, heat in boiling water for 15 min, and immediately cool in running water to 25°C or lower.
- (5) Take the cap off, add 2 ml of potassium iodide solution and 2 ml of sulfuric acid (250 g/l), and immediately titrate with 0.05 mol/l sodium thiosulfate solution. Add 1 to 2 drops of starch solution as an indicator near the end point at which a little yellow remains, titrate until the blue disappears, and obtain the titration value (f) (ml).
- (6) Perform the operation of (1) to (5) by inverting the additional order of the Fehling liquid B and the sample solution separately as a blank test, and obtain the titration value (g) (ml).
- (7) Calculate the quantity of glucose generated from the titration value from the following formula.

$$e = (g - f) \times 1.62 \times f$$

where, e : amount of generated glucose (mg)

f : titration value (ml)

g : titration value in blank test (ml)

1.62 : glucose quantity equivalent to 1 ml of 0.05 mol/l sodium thiosulfate solution (mg)