

Test Method for Bacterial Alpha-Amylase Enzymes used in Desizing

1. Purpose and Scope

1.1 This test method is intended for the assay of the bacterial amylases employed commercially for textile desizing. It is not applicable to products that contain *beta*-amylase in addition to *alpha*-amylase.

2. Principle

2.1 Dextrinogenic amylase activity is measured in terms of the digestion time required to produce a color change denoting a definite stage of dextrinization of the starch substrate. The amylase content of the sample, expressed in Bacterial Amylase Units (BAU), is readily calculated from the dextrinizing time (see 13.1).

3. Terminology

3.1 **Bacterial Amylase Unit (BAU), n.**—a measure of starch degradation as shown by the quantity of an enzyme that will dextrinize one milligram of starch per minute under the specified experimental conditions.

4. Safety Precautions

NOTE: These safety precautions are for information purposes only. The precautions are ancillary to the testing procedures and are not intended to be all inclusive. It is the user's responsibility to use safe and proper techniques in handling materials in this test method. Users MUST consult manufactures for specific details such as safety data sheets and other recommendations. Consult and follow all applicable OSHA standards and rules.

4.1 Follow good laboratory practices. Wear safety glasses in all laboratory areas.

4.2 Handle all chemicals with care. Use chemical goggles or face shield, impervious gloves and an impervious apron during dispensing and mixing of iodine; this is a corrosive chemical.

4.3 Ensure an eyewash/safety shower is located nearby and a self-contained breathing apparatus is readily available for emergency use.

4.4 Control exposure to chemicals used in this procedure at or below levels set by governmental authorities [e.g., Occupational Safety and Health Administration's (OSHA) permissible exposure limits

(PEL) as found in 29 CFR 1910.1000; see web site: www.osha.gov for latest version]. In addition, the American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values (TLVs) comprised of time weighted averages (TLV-TWA), short term exposure limits (TLV-STEL) and ceiling limits (TLV-C) are recommended as a general guide for air contaminant exposure which should be met (see 13.2).

5. Uses and Limitations

5.1 The calibration of the method as presented here further restricts its use to amylases of bacterial origin.

5.2 Some surface-active agents interfere with the color development which is an essential part of this method. The method is therefore not suitable for assessing the effect of surfactants on enzymes.

6. Apparatus (see 13.3)

6.1 Comparator (see 13.4).

6.2 Water bath, controlled at constant temperature $30 \pm 1^\circ\text{C}$ ($86 \pm 2^\circ\text{F}$).

6.3 Electric timer.

6.4 Glassware (see 13.5).

6.4.1 Borosilicate test tubes, 13×100 mm—at least six dozen should be on hand.

6.4.2 Standard volumetric pipettes to deliver 2 mL, 5 mL, 10 mL, 20 mL, fast 1 mL blow-out pipette for sampling hydrolyzing mixture, plus assorted standard pipettes to accomplish necessary sample preparation dilutions.

7. Reagents

7.1 Iodine (crystalline); reagent grade; I_2

7.2 Potassium iodide; KI

7.3 Potassium dihydrogen phosphate; KH_2PO_4 (monobasic)

7.4 Sodium phosphate; Na_2HPO_4 (dibasic)

7.5 Merck's Lintner Starch

8. Preparations

8.1 Test Sample Preparation.

8.1.1 Prepare the sample solution by dilution with water so that 10 mL of the final solution (see Table I) will give a

Table I—Guide to Selection of Sample Weight

Sample alpha-amylase content (BAU per g)	Sample weight to be used mg sample per 10 mL final dilution
70-250	200
125-500	100
300-900	50
600-1800	25
1000-4000	10
3000-9000	5
6000-18000	2.5
12500-50000	1

dextrinizing time of 15-35 min. With dry samples, insoluble materials may be present but it is generally unnecessary to filter the solution.

8.1.2 Table I shows sample weight to be used with samples of varying alpha-amylase content. If the sample can be placed in either of two ranges, it is preferable to use the sample weight corresponding to the higher range since this will give a longer dextrinization time, making for easier and more accurate measurements.

8.1.3 Liquid products should be weighed as the specific gravity is usually greater than 1.0.

8.1.4 In all cases, a sufficient amount of sample should be weighed to minimize weighing errors. If necessary, the amount of sample to be applied to the test may be obtained by secondary volume dilution.

8.2 Stock Iodine Solution.

8.2.1 Weigh in glass, 5.5 g reagent grade crystalline iodine; to dissolve, add 11 g reagent grade potassium iodine dissolved in minimal (10-12 mL) water. When iodine has completely dissolved dilute to 250 mL.

8.2.2 Preserve this solution in an amber glass-stoppered bottle and store under refrigeration. Solution may be used for three (3) months.

8.3. Dilute Iodine Solution.

8.3.1 Dissolve 2.0 mL of stock iodine solution and 20 g reagent grade potassium iodide in water, and dilute to 500 mL.

8.3.2 The dilute iodine solution may be stored under refrigeration and used for one (1) week. However, at the time of its use (see 9.1), it should be at $30 \pm 1^\circ\text{C}$ (86

± 2°F).

8.4 Buffer Solution, pH 6.6.

8.4.1 Solution A. Dissolve 9.078 g of KH_2PO_4 in water and dilute to 1 L.

8.4.2 Solution B. Dissolve 9.472 g of Na_2HPO_4 in water and dilute to 1 L.

8.4.3 Mix 600 mL of Solution A and 400 mL of Solution B to obtain pH 6.6 buffer.

8.5 Buffered Starch Substrate.

8.5.1 Determine the dry weight of Merck's Lintner Starch (Special for Diastatic Power Determinations) by drying 20.00 g of starch at 103-104°C (217-219°F) for 3 h. Weigh after cooling in desiccator and continue drying until weight is constant. Discard after dry weight has been determined.

8.5.2 Calculate the amount of starch equivalent to 10.00 g dry basis for 500 mL starch substrate.

8.5.3 Starch should be retained in tightly closed jar and not exposed to environment where the moisture content would be subject to change.

8.5.4 Quantitatively transfer a slurry of 10.00 g (dry weight basis) of Merck's Lintner Starch into about 300 mL of vigorously stirred boiling water contained in a 1-L Pyrex beaker. Leave the stirring rod in the solution after boiling resumes and boil for exactly 3 min.

8.5.5 Cool to room temperature in a cold-water bath with continuous stirring to avoid skin formation (surface dehydration).

8.5.6 Quantitatively transfer the starch into a 500-mL volumetric flask using a small quantity of water to complete the transfer.

8.5.7 Add 10 mL of pH 6.6 buffer and dilute to the mark.

8.5.8 Check the pH of the starch substrate with a standardized pH meter.

8.5.9 Starch substrate should be free of lumps or flakes and prepared fresh daily. Contamination of the starch substrate with even minute traces of enzyme will render the substrate unsuitable for use.

9. Procedure

9.1 Dispense 5.0 mL dilute iodine solution in each of a series of test tubes and allow to temperate in the $30 \pm 1^\circ\text{C}$ ($86 \pm 2^\circ\text{F}$) bath.

9.2 Transfer 20.0 mL of buffered starch substrate into a 50-mL Erlenmeyer (or equivalent) (lead rings are convenient to weight the flask), stopper, place in $30 \pm 1^\circ\text{C}$ ($86 \pm 2^\circ\text{F}$) bath allowing about 15 min to attain uniform temperature within the flask.

9.3 Likewise, temperate an appropriate amount of freshly prepared sample solution. Rapidly add 10.0 mL of the sample solution, using a blow-out type pipette, and start the timer. After pipette has drained, replace rubber stopper and swirl

flask vigorously to ensure proper mixing.

9.4 At appropriate time intervals, add 1 mL (blow-out pipette with cotton plug) of the hydrolyzing mixture to 5 mL of dilute iodine solution at $30 \pm 1^\circ\text{C}$ ($86 \pm 2^\circ\text{F}$), shake to mix thoroughly, pour into the 13 mm precision square tube, and compare with standard alpha-amylase color disc in the comparator. After completing the comparison, empty the square tube, giving it a quick shake so that very little liquid remains. The tube may now be used for another test.

9.5 During the initial stages of reaction, it is not necessary that the 1-mL sample be measured precisely before adding to the dilute iodine solution. As the end point is approached, the addition must be made accurately. The contents of the pipette are blown into the iodine solution in order that the time may be more accurately measured.

9.6 Around the time of the end point, samples should be taken every 0.5 min. In case two (2) samples 0.5 min apart show that one is darker than the standard color and the other one is lighter, record the end-point time as the quarter minute between these two times.

9.7 Care must be exercised to avoid contact of the 1-mL hydrolyzing pipette with the dilute iodine solution. A carry-back of iodine to the hydrolyzing mixture will interfere with enzyme action.

10. Calculation

10.1 Calculate the alpha-amylase content of the sample by using the following equation:

$$\text{BAU per g} = 40(F)/T$$

10.2 Explanation of equation (see 13.1):

BAU = Bacterial Amylase Units per gram (g)

F = Dilution Factor (total dilution volume/sample weight in grams)

T = Dextrinizing time in minutes

10.3 The preceding equation follows from:

(a) the definition of the BAU as the quantity of enzyme that dextrinized one mg of starch per minute, and

(b) the assay practice of dextrinizing 400 mg of starch (20 mL of 2% solution) with a 10 mL aliquot of enzyme solution. Thus:

$$\text{BAU per gram} = 400/T \times F/10 = 40F/T$$

10.4 Example of Calculations:

10.4.1 If we assume that for a particular sample being tested the anticipated BAU/g is 800, reference to Table I indicates that 25 mg of sample per 10 mL

of final dilution should be employed. Therefore, 2.5 g of the sample would be weighed out, and diluted to 1000 mL. It now becomes possible to calculate the Dilution Factor, F :

$$F = \text{Total Dilution Volume/Sample Weight in grams} = 1000/2.5 = 400$$

10.4.2 If the dextrinizing time is 20 min, the BAU/g can be calculated:

$$\text{BAU/g} = 40 F/T = 40 \times 400/20 = 16000/20 = 800$$

10.4.3 If BAU does not fall within expected limits, a retest should include the practice of making up an entirely new sample preparation. This constitutes a check on the preparation of the test solution where errors may have occurred resulting in erroneous BAU.

11. Report

11.1 Report the amylase content of the sample as Bacterial Amylase Units (BAU) per gram.

12. Precision and Bias

12.1 *Precision*. The mean of duplicate tests should check within $\pm 6.5\%$ of the true mean at the 95% confidence level. This information is based on an interlaboratory study.

12.2 *Bias*. The values, Bacterial Assay Units (BAU), can only be defined in terms of a test method. There is no independent method for determining the true value. Based on the information available, the method has no known bias.

13. Notes

13.1 If approximate or anticipated BAU of material to be tested is known, determine dilution factor (F) by multiplying selected test time (T) by anticipated BAU and dividing by 40, thus: $T \times \text{BAU}/40 = F$.

13.2 Available from Publications Office, ACGIH, Kemper Woods Center, 1330 Kemper Meadow Dr., Cincinnati OH 45240, USA; +1. 513.742.2020; www.acgih.org.

13.3 For potential apparatus, reagents or materials sources, visit the AATCC Buyer's Guide at www.aatcc.org/bg. AATCC provides its Corporate members the option to their items and services. AATCC does not qualify, or in any way approve, endorse or certify that any of the listings meet the specifications in its standards.

13.4 Light Source. Should be either daylight or daylight fluorescent lamps. Incandescent lamps should not be used since they give slightly lower results.

13.5 All glassware must be clean. This applies particularly to pipettes where a hang-up of droplets interferes with correct volume delivery. Sulfuric acid-potassium dichromate