



Association of Textile, Apparel & Materials Professionals

AATCC takes no position respecting the validity of any patent rights asserted in connection with any item mentioned in this test method. Users of this test method are expressly advised that determination of the validity of any such patent rights, and the risk of infringement of such rights, are entirely their own responsibility.

This test method is subject to revision at any time by the responsible technical committee and must be reviewed every five years and if not revised, either reaffirmed or withdrawn. Your comments are invited either for revision of this test method or for additional procedures and should be addressed to the AATCC Technical Center. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend. If you feel that your comments have not received a fair hearing, you should make your views known to Diana A. Wyman, Technical Director, at the address shown below.

This test method is copyrighted by AATCC, PO Box 12215, Research Triangle Park, NC 27709 USA. Individual reprints (single or multiple copies) of this method may be obtained by contacting AATCC at the above address or tel: 919-549-3526; fax: 919-549-8933, or e-mail: orders@aatcc.org.

AATCC License Agreement: This test method is copyrighted by the American Association of Textile Chemists and Colorists (AATCC), PO Box 12215, 1 Davis Drive, Research Triangle Park, NC USA. All rights reserved.

AATCC grants you a license as follows: The right to download one electronic file of this AATCC test method for temporary storage on one computer for purposes of viewing, and/or printing one copy of the AATCC test method for individual use. Neither the electronic file nor the hard copy print may be reproduced in any way. In addition, the electronic file may not be distributed elsewhere over computer networks or otherwise. The hard copy print may only be distributed to other employees for their internal use within your organization.

This test method is not for resale.

Copyright The American Association of Textile Chemists and Colorists

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 Dextrogenic 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 manufacturers 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 dextrinizing time of 15-35 min. With dry samples, insoluble materials may be

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

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 $30 \pm 1^\circ\text{C}$ ($86 \pm 2^\circ\text{F}$).

8.4 Buffer Solution, pH 6.6.

8.4.1 Solution A. Dissolve 9.078 g of