



Designation: D3048 – 89 (Reapproved 2003)

Standard Test Method of Assay for Alkaline Protease¹

This standard is issued under the fixed designation D3048; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ϵ) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers the assay of alkaline protease enzymes. This procedure is applicable to enzyme preparations with high activity but is inapplicable to formulated detergent products or air samples.

1.2 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* Material Safety Data Sheets are available for reagents and materials. Review them for hazards prior to usage.

2. Referenced Documents

2.1 *ASTM Standards:*²

D459 Terminology Relating to Soaps and Other Detergents³

D1129 Terminology Relating to Water³

D1193 Specification for Reagent Water⁴

E131 Terminology Relating to Molecular Spectroscopy

3. Terminology

3.1 *Definitions:*

3.1.1 *APB unit*—that amount of enzyme which releases in 1 min under the conditions of the test a casein hydrolysate that has the same absorbance as 1 μg of tyrosine in an equivalent volume. The number of APB units per gram of a preparation is called the APB of the preparation.

3.1.2 *standardized enzyme*—an enzyme preparation of known activity for calibrating the sample enzyme in terms of a gravimetric standard of enzymatic activity.⁵

3.1.3 The terms “alkyl benzene sulfonate (ABS)” and “linear alkylate sulfonate (LAS)” in this method are defined in accordance with Terminologies D1129 and D459D1129D459:

3.1.3.1 *alkyl benzene sulfonate (ABS)*—the generic name applied to the neutralized product resulting from the sulfonation of an alkylated benzene.

3.1.3.2 *linear alkylate sulfonate (LAS)*—a form of alkyl benzene sulfonate (ABS) in which the alkyl group is linear rather than a branched chain.

3.1.4 *nonionic surfactant*—a mixed C₁₆-C₁₈ linear primary alcohol containing 65 % ethylene oxide.

3.1.5 For definitions of other terms used in these methods, refer to Terminology E131.

4. Summary of Test Method

4.1 This test is based on the hydrolysis of casein at 50°C for 15 min at pH 9. The trichloroacetic acid-soluble hydrolysate is assayed by the spectrophotometric determination of the absorbance at approximately 275 nm.⁶ The results are correlated with the absorptivity of tyrosine or the absorbance of hydrolysate from standardized enzyme. Results are reported as APB, which is defined in Section 3, or in micrograms of pure crystalline enzyme per gram of sample.

5. Apparatus

5.1 *Water Bath*, constant-temperature, maintained at 50 \pm 0.2°C.

5.2 *Ultraviolet Spectrophotometer*, suitable for liquid measurements at a wavelength of approximately 275 nm.

5.3 *Absorption Cell*, silica, 10-mm light path.

5.4 *pH Meter*.

5.5 *Test Tubes*, 25 by 150 mm.

6. Reagents

6.1 *Purity of Reagent*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.⁷ Other grades may be used, provided it is first ascertained that the reagent is of

¹ This test method is under the jurisdiction of ASTM Committee D12 on Soaps and Other Detergents and is the direct responsibility of Subcommittee D12.12 on Analysis of Soaps and Synthetic Detergents.

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² *Annual Book of ASTM Standards*, Vol 15.04.

³ *Annual Book of ASTM Standards*, Vol 11.01.

⁴ *Annual Book of ASTM Standards*, Vol 03.06.

⁵ Available from National Institute of Occupational Safety and Health, 1014 Broadway Ave., Cincinnati, Ohio 45202.

⁶ Bailey, J. L. “Techniques in Protein Chemistry,” Elsevier Publishing Co., New York, NY. Chapter 11, 1967, pp. 340–352.

⁷ *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.