



Standard Test Method for The Analysis of Aqueous Extractable Protein in Natural Rubber and Its Products Using the Modified Lowry Method¹

This standard is issued under the fixed designation D 5712; 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 an analytical test for determining the amount of total aqueous extractable protein associated with natural rubber (NR) and its products. Water soluble proteins are extracted in a buffer solution and then precipitated to concentrate them and also to separate them from water soluble substances which may interfere with the determination. The extracted protein is redissolved and quantified colorimetrically by the modified Lowry method using a protein standard.

1.2 For the purpose of this test method, the range of protein measurement will be based on the limit of detection and quantitation and recorded in micrograms per dm² NR specimen.

1.3 The test method is designed to be accurate and compatible with the industrial environment.

1.4 Steps are included in this test method to minimize the effects of interfering substances.

1.5 It is recognized that other methods for the analysis of leachable proteins exist and these may be used for routine quality control purposes provided they have been validated and a correlation established against the reference method specified by this Standard.

1.6 The values stated in SI units are to be regarded as the standard.

1.7 *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.*

2. Referenced Documents

2.1 ASTM Standards:

D 3577 Specification for Rubber Surgical Gloves²

D 3578 Specification for Rubber Examination Gloves²

D 4483 Practice for Determining Precision for Test Method Standards in the Rubber and Carbon Black Industries³

¹ This test method is under the jurisdiction of ASTM Committee D-11 on Rubber and is the direct responsibility of Subcommittee D11.40 on Consumer Rubber Products.

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² Annual Book of ASTM Standards, Vol. 09.02.

³ Annual Book of ASTM Standards, Vol. 09.01.

3. Terminology

3.1 Definitions:

3.1.1 *background*—the absorbance measurement of the Lowry assay in the absence of the protein analyte.

3.1.2 *calibration*—the standardization of an instrument setting.

3.1.3 *calibration solution*—the standard solution used to routinely and reproducibly calibrate a measuring instrument.

3.1.4 *dilution factor (F)*—the ratio of the volume NaOH in mL used to redissolve the test specimen extract to volume NaOH in mL used to redissolve the standard ovalbumin proteins. For example, if protein in a 1 mL test extract is acid precipitated and redissolved in 0.25 mL, and the ovalbumin protein standards are also redissolved in 0.25 mL, then the dilution factor ratio of the test extract to that of the calibration curve would equal one.

3.1.5 *concentration range*—the recommended analyte concentration range in µg/mL that produces an absorbance measurement of 0.01 to 1.5 units at 600 to 750 nm.

3.1.6 *extractant*—an aqueous buffer of pH 7.4 ± 0.2 used for the extraction process.

3.1.7 *initial setting*—the instrument setting to which the spectrophotometer is adjusted with the reference solution.

3.1.8 *interferent*—any substance which results in a false positive or negative measurement in the analytical test method.

3.1.9 *latex protein*—aqueous extractable proteins and polypeptides occurring in NR latex and its products.

3.1.10 *limit of detection (LOD)*—the lowest protein concentration that can be measured and be statistically different from the blank. The LOD is expressed as 3.3 × standard error of the y-intercept of the calibration regression line divided by the slope of the calibration line.

3.1.11 *limit of quantitation (LOQ)*—the lowest protein concentration that can be measured to produce quantitatively meaningful results with acceptable precision and accuracy. The LOQ is expressed as 10 × standard error of the y-intercept of the calibration regression line divided by the slope of the calibration line.

3.1.12 *linearity*—the degree to which a graph of absorbance versus concentration approximates a straight line.

