This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

Designation: D6499 – 24

Standard Test Method for Immunological Measurement of Antigenic Protein in Hevea Natural Rubber (HNR) and its Products[1]

This standard is issued under the fixed designation D6499; 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 immunological method to determine the amount of antigenic protein in Hevea Natural Rubber and its products using rabbit antisera specific for HNRL proteins. This immunoassay procedure quantitatively measures the level of antigenic latex proteins in solution using an inhibition format. The samples may include glove or other product extracts which have been collected in order to measure the HNR levels. Although this method detects antigenic proteins, it should not be considered as a measure of allergenic proteins. Correlation of protein/antigen levels with the level of allergenic proteins has not been fully established.

1.2 For the purpose of this test method, the range of protein will be measured in terms of microgram to milligram quantities.

1.3 This test method can be used to determine the presence and amount of HNR protein contamination on test items.

1.4 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.5 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D4483 Practice for Evaluating Precision for Test Method Standards in the Rubber and Carbon Black Manufacturing Industries

D5712 Test Method for Analysis of Aqueous Extractable Protein in Latex, Natural Rubber, and Elastomeric Products Using the Modified Lowry Method

E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

3. Terminology

3.1 *Definitions:*

3.1.1 *allergens, n*—protein antigens which induce allergic immune reactions typically mediated through IgE antibodies.

3.1.2 *antibody, n*—an immunoglobulin, a protein that is produced as a part of the immune response which is capable of specifically combining with the antigen.

3.1.3 *antigen, n*—any substance that provokes an immune response when introduced into the body.

3.1.4 *background absorbance, n*—the absorbance reading in the solution resulting from the presence of chemicals, ions etc. other than the substrate being determined.

3.1.5 *blocking solution, n*—a non-reactive protein solution used to prevent nonspecific antibody adsorption.

3.1.6 *calibration, n*—the standardization of an instrument setting or an assay configuration.

3.1.7 *concentration range, n*—the recommended analyte concentration range in µg/mL that produces an absorbance reading of 0.1 to 2.0 units.

3.1.8 *enzyme linked immunosorbent assay (ELISA), n*—an immunological test method to quantify antigen or antibody levels using an enzyme as the detection mechanism.

3.1.9 *primary antibody, n*—the antibody used first in a sequence that is specific for the antigen.

3.1.10 *reference solution, n*—the solution to which the test sample is being compared against.

3.1.11 *repeatability, n*—the variability or test error between independent test results obtained within a single laboratory.

[1] This test method is under the jurisdiction of ASTM Committee D11 on Rubber and Rubber-like Materials, and is the direct responsibility of Subcommittee D11.40 on Consumer Rubber Products.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at www.astm.org/contact. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

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