



National Institute of Standards & Technology

Report of Investigation

Reference Material 8323

Yeast Protein Extract

This Reference Material (RM) 8323 is intended to support measurements used to identify proteins in complex protein mixtures such as those used in proteomics. RM 8323 can be used to help assess measurement repeatability within a laboratory or comparability between laboratories or among different measurement approaches [1]. RM 8323 can also be used in the development and validation of new measurement approaches for identifying proteins in complex protein mixtures. A unit of RM 8323 consists of three vials, each containing 200 μL of frozen yeast protein extract solution. The proteins extracted from *Saccharomyces cerevisiae* have been solubilized in 50 mmol/L ammonium bicarbonate in water.

RM 8323 was developed in collaboration with the Clinical Proteomic Technologies for Cancer (CPTC) initiative of the National Cancer Institute. The proteome from *S. cerevisiae* was used in several CPTC interlaboratory studies that aimed to assess the repeatability and reproducibility of proteomic measurement for protein identification [2,3].

Reference Total Protein Concentration: A reference mass concentration value for total protein is provided in Table 1. A NIST reference value [4] is a noncertified value that is the best estimate of the true value; however, the value does not meet NIST criteria for certification and is provided with associated uncertainties that may reflect only measurement precision and may not include all sources of uncertainty. A spectrophotometric method employing bicinchoninic acid (BCA) was utilized for total protein concentration measurements [5]. SRM 927e *Bovine Serum Albumin (7% Solution)* was used to prepare calibration solutions.

Table 1. Reference Concentration and Uncertainty for Total Protein^(a)

0.188 mg/mL \pm 0.038 mg/mL

^(a) The reference concentration value for total protein is the consensus mean of measurements from a single NIST method. The uncertainty in the reference value, calculated according to the method described in the ISO/JCGM Guide [6], incorporates Horn-Horn-Duncan (HHD) uncertainty for the Type A component. The measurand is the mass concentration value listed based on the method used. Metrological traceability is to the SI derived unit for mass concentration (expressed as milligrams per milliliter).

Expiration of Value Assignment: RM 8323 is valid, within the measurement uncertainty specified, until **01 April 2023**, provided the RM is handled and stored in accordance with the instructions given in this Report of Investigation (see “Instructions for Storage and Use”). The report is nullified if the RM is damaged, contaminated, or otherwise modified.

Maintenance of RM: NIST will monitor this RM over the period of its validity. If substantive technical changes occur that affect the value assignment before expiration of this report, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Overall direction and coordination of technical measurements leading to value assignment were performed by A. Beasley Green, D.M. Bunk and K.W. Phinney of the NIST Biomolecular Measurement Division.

Analyses were performed by A. Beasley Green and D.M. Bunk.

Statistical consultation and analysis was performed by A. Heckert of the NIST Statistical Engineering Division.

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Support aspects involved in the preparation of this RM were coordinated through the NIST Office of Reference Materials.

NOTICE AND WARNING TO USER

RM 8323 has been obtained from yeast cells and has the potential to contain toxins that may pose a health risk. Normal caution and care should be exercised during the material's handling and use.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The yeast protein extract solution is shipped frozen (on dry ice) and, upon receipt, should be stored frozen until ready for use. A freezer temperature of $-20\text{ }^{\circ}\text{C}$ is acceptable for storage for up to one week. If a longer storage time is anticipated, the material should be stored at or below $-60\text{ }^{\circ}\text{C}$. The RM should not be exposed to sunlight or ultraviolet radiation. Storage of thawed material at room or refrigerator temperatures may result in degradation or modification of constituent proteins.

Use: Vials of the RM to be analyzed should be removed from the freezer and allowed to stand at room temperature ($20\text{ }^{\circ}\text{C}$ to $25\text{ }^{\circ}\text{C}$) until thawed. After the material is thawed, it should be used immediately. The material should be mixed briefly with a vortex mixer before aliquots are withdrawn.

PREPARATION AND ANALYSIS⁽¹⁾

Material Acquisition and Preparation: Lysate from *S. cerevisiae* was obtained from Boston Biochem Inc. (Cambridge, MA). The *S. cerevisiae*, strain BY4741, was grown in a 100 L batch of rich (yeast peptone dextrose) medium at $30\text{ }^{\circ}\text{C}$ in a fermentor until an optical density of approximately 0.93 was reached. The yeast was harvested by continuous-flow centrifugation, and the cell pellet was then washed twice with ice-cold water. The cells were lysed by incubation with ice-cold trichloroacetic acid (10 mL/L) in water for 1 h at $4\text{ }^{\circ}\text{C}$. The precipitate was collected by centrifugation, washed twice with 100 mL/L water in acetone, and pelleted again.

The lyophilized yeast lysate was homogenized at NIST through manual grinding. The ground yeast lysate powder was suspended in 50 mmol/L ammonium bicarbonate containing 6 mol/L urea in water, pH 7.85. After gently stirring at $5\text{ }^{\circ}\text{C}$ overnight, the yeast lysate solution was filtered through a $0.22\text{ }\mu\text{m}$ cellulose acetate filter. To remove urea from the yeast lysate solution, the solution was thoroughly dialyzed (6,000 Da to 8,000 Da cutoff) at $5\text{ }^{\circ}\text{C}$ using 50 mmol/L ammonium bicarbonate in water as the dialysis buffer.

Homogeneity Analysis: The homogeneity was assessed at the time the analyses for the reference value were performed. A stratified random sampling plan was devised to test for homogeneity across the production lot. The results indicated that there was no apparent trend in the data when plotted against the sequence in which the vials were prepared.

⁽¹⁾ Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

REFERENCES

- [1] Beasley-Green, A.; Bunk, D.M.; Rudnick, P.A.; Kilpatrick, L.; Phinney, K.W.; *Proteomics*, Vol. 12, pp. 923–931 (2012).
- [2] Paulovich, A.G.; Billheimer, D.; Ham, A-J.L.; Vega-Montoto, L.J.; Rudnick, P.A.; Tabb, D.L.; Wang, P.; Blackman, R.K.; Bunk, D.M.; Cardasis, H.L.; Clauser, K.R.; Kinsinger, C.R.; Schilling, B.; Tegeler, T.J.; Variyath, A.M.; Wang, M.; Whiteaker, J.R.; Zimmerman, L.J.; Fenyó, D.; Carr, S.A.; Fisher, S.J.; Gibson, B.W.; Mesri, M.; Neubert, T.A.; Reginier, F.E.; Rodriguez, H.; Spiegelman, C.; Stein, S.E.; Tempst, P.; Liebler, D.C.; *Mol. Cell. Proteomics*, Vol. 9, pp. 242–254 (2009).
- [3] Tabb, D.L.; Vega-Montoto, L.; Rudnick, P.A.; Variyath, A.M.; Ham, A-J.L.; Bunk, D.M.; Kilpatrick, L.E.; Billheimer, D.D.; Blackman, R.K.; Cardasis, H.L.; Carr, S.A.; Clauser, K.R.; Jaffe, J.D.; Kowalski, K.A.; Neubert, T.A.; Regnier, F.E.; Schilling, B.; Tegeler, T.J.; Wang, M.; Wang, P.; Whiteaker, J.R.; Zimmerman, L.J.; Fisher, S.J.; Gibson, B.W.; Kinsinger, C.R.; Mesri, M.; Rodriguez, H.; Stein, S.E.; Tempst, P.; Paulovich, A.G.; Liebler, D.C.; Spiegelman, C.; *J. Proteome. Res.*, Vol. 9, pp. 761–776 (2009).
- [4] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136; U.S. Government Printing Office: Washington, DC (2000); available at: <https://www.nist.gov/srm/publications.cfm> (accessed Feb 2018).
- [5] Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N. M., Olson, B. J., and Klenk, D. C. *Anal. Biochem.* Vol. 150, pp. 76-85 (1985).
- [6] JCGM 100:2008; *Guide to the Expression of Uncertainty in Measurement*; (GUM 1995 with Minor Corrections), Joint Committee for Guides in Metrology (JCGM) (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Feb 2018); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at <https://physics.nist.gov/Pubs/contents.html> (accessed Feb 2018).

Report Revision History: 20 February 2018 (Revised total protein value based on re-evaluation of the original analytical results and updated the entire report to current NIST standards; changed expiration date; editorial changes); 09 July 2010 (Original report date).

Users of this RM should ensure that the Report of Investigation in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-2200, fax (301) 948-3730, e-mail srminfo@nist.gov, or via the Internet <https://www.nist.gov/srm>.