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Reference Material 8323
Yeast Protein Extract
REFERENCE MATERIAL INFORMATION SHEET

Purpose: 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.

Description: 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.

Non-Certified Values: Non-certified values are suitable for use in method development, method harmonization, and process control but do not provide metrological traceability to the International System of Units (SI) or other higher-order reference system. A spectrophotometric method employing bicinchoninic acid (BCA) was utilized for total protein content measurements [4]. Non-certified mass concentration value for total protein content is provided below.

Material	Mass Concentration[(a)] (mg/mL)
RM 8323 Total Protein Content	0.188 ± 0.038

[(a)] Values are expressed as $x \pm U_{95\%}(x)$, where x is the non-certified value and $U_{95\%}(x)$ is the expanded uncertainty of the non-certified value. The non-certified value for total protein content is the consensus mean of measurements from a single NIST method. The uncertainty in the non-certified value, calculated according to the method described in the ISO/JCGM Guide [5], incorporates Horn-Horn-Duncan (HHD) uncertainty for the Type A component.

Period of Validity: The non-certified values are valid within the measurement uncertainty specified until **01 March 2028.** The value assignments are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Non-Certified Values: NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Reference Material Information Sheet and notify registered users. RM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the RM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (https://www.nist.gov/srm).

Michael Tarlov, Chief
Biomolecular Measurement Division

Steven J. Choquette, Director
Office of Reference Materials

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Safety: RM 8323 is intended for research use; not for human consumption. 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.

Storage: The original unopened bottles of RM 8323 is shipped frozen (on dry ice) and, upon receipt, should be stored frozen until ready for use. A freezer temperature of –20 °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 °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: Before use, the contents of the unopened bottle should be mixed thoroughly by inverting and/or rolling. Vials of the RM to be analyzed should be removed from the freezer and allowed to stand at room temperature (20 °C to 25 °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.

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.; Fenyo, 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] 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).

[5] 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 https://www.bipm.org/en/committees/jc/jcgm/publications (accessed Dec 2022); 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.

If you use this RM in published work, please reference:

Beasley-Green, A.; Bunk, D.M.; Rudnick, P.A.; Kilpatrick, L.; Phinney, K.W.; *Proteomics*, Vol. 12, pp. 923–931 (2012).

Information Sheet Revision History: **21 December 2022** (Changed expiration date; updated format; editorial changes); **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 certificate date).

Certain commercial equipment, instruments, or materials may be identified in this Reference Material Information Sheet to adequately specify 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.

Users of this RM should ensure that the Reference Material Information Sheet in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at https://www.nist.gov/srm.

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APPENDIX A

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.

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

Source 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 °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 °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 ºC overnight, the yeast lysate solution was filtered through a 0.22 µm 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 °C using 50 mmol/L ammonium bicarbonate in water as the dialysis buffer.

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

Analysis: Value assignment of the mass concentration of total protein content in RM 8323 was based on the measurements made by NIST using a spectrophotometric method employing bicinchoninic acid (BCA) for determination of total protein content [4]. SRM 927e *Bovine Serum Albumin (7% Solution)* was used to prepare calibration solutions.

Homogeneity Analysis: The homogeneity was assessed at the time the analyses for the non-certified 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.

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