NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
U.S. DEPARTMENT OF COMMERCE

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Standard Reference Material® 2393

CAG Repeat Length Mutation in Huntington's Disease

CERTIFICATE OF ANALYSIS

Purpose: The certified values delivered by this Standard Reference Material (SRM) are intended for use in the value assignment of the number of Huntington's disease [CAG] trinucleotide repeats contained in a human genomic material [1–3]. The six component genomic DNA materials, labeled A to F, are from DNA extracted from cell lines derived from Huntington's disease samples.

Description: A unit of SRM 2393 consists of six sterile 0.5 mL perfluoroalkoxy (PFA) fluoropolymer vials, each vial containing approximately 50 µL of DNA solution of each individual component.

Certified Values: A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated. The certified value of the number of CAG repeats is traceable to the SI unit of count [4]. These certified values were determined by counting the number of [CAG] repeat units in the component alleles by Sanger sequencing technology and confirmed by fragment analysis (genotyping). The complete agreement among results from independent forward and the reverse direction sequencing and fragment analysis provides the highest confidence in the assigned integer counts. The certified number of [CAG] repeats for each component is provided below.

Component	Allele 1	Allele 2
A	15	29
B	17	36
C	15	40
D	35	45
E	39	50
F	17	75[(a)]

[(a)] Template slippage ("stutter") may result in the PCR product Peak area and/or height not being highest for the parent allele, as is observed for the 75 repeat for the parent allele (See Figure 1 in Appendix A).

Additional Information: Additional information is provided in Appendix A. Values of potential interest to users are provided in Appendix B.

Period of Validity: The certified values delivered by **SRM 2393** are valid within the measurement uncertainty specified until **31 January 2031**. The certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Certified Values: NIST will monitor this SRM over the period of its validity. If substantive technical changes occur that affect the certification, NIST will issue an amended certificate through the NIST SRM website (https://www.nist.gov/srm). 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).

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Certificate Revision History on Page 2

Safety: SRM 2393 is a human source material. SRM 2393 is a Biosafety Level 1 material and should be handled according to applicable federal, state, and/or local regulations and according to policies and procedures of recipient's organization.

Storage: All vials of SRM 2393 should be stored in the dark between 2 °C to 8 °C. **DO NOT FREEZE.**

Use: Component vials should be mixed briefly and centrifuged (without opening the vial cap) prior to removing sample aliquots for analysis. Materials should be withdrawn immediately after opening the vials and processed without delay. Dilutions of these materials may be made as appropriate, but they must be used immediately. Certified values and values of potential interest to users do not apply to any material remaining in recapped vials. **DO NOT EXPOSE ANY DNA SOLUTION TO DIRECT SUNLIGHT**.

REFERENCES

[1] Bean, L.; Bayrak-Toydemir, P.; Technical Standards and Guidelines for Huntington Disease; *Standards and Guidelines for Clinical Genetics Laboratories*; American College of Medical Genetics and Genomics: Bethesda, MD (2014); available at https://www.acmg.net/PDFLibrary/Huntington-Disease.pdf (accessed Aug 2025).

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[4] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Duewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Molloy, J.; Nelson, M.A.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sander, L.C.; Schiel, J.E.; Sharpless, K.E.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication 260-136, 2021 edition; National Institute of Standards and Technology, Gaithersburg, MD (2021); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf (accessed Aug 2025).

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[7] European Huntington's Disease Network Registry; available at http://www.ehdn.org/ (accessed Aug 2025).

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[9] Lee, K.M.; Choi, K.H.; Ouellette, M.M; *Use of Exongenous hTERT to Immortalize Primary Human Cells*; Cytotechnology, Vol. 45, pp. 33–38 (2004).

Certificate Revision History: 04 Aug 2025 (Change of period of validity; editorial changes; format update); **14 August 2020** (Change of expiration date; editorial changes); **01 October 2015** (Change of expiration date; editorial changes); **17 December 2012** (Reference laboratory information updated; editorial changes); **03 March 2011** (Original certificate issue date).

Certain commercial equipment, instruments, or materials may be identified in this Certificate of Analysis 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 SRM should ensure that the Certificate of Analysis 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

Sample Preparation: DNA extracts from the National Institute of General Medical Sciences (NIGMS) Human Genetic Cell Repository, Inherited Disorders subcollection [5] were purchased from Coriell Institute for Medical Research, (Camden, NJ.) The purchased materials were solubilized in deionized water with 10 mmol/L 2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride (Tris HCl) and 0.1 mmol/L ethylenediaminetetra-acetic acid disodium salt (disodium EDTA) adjusted to pH 8.0 (TE^{-4}, pH 8.0 buffer). After concentration adjustment the materials were allowed to equilibrate at 4 °C in PFA containers until vialing. Just prior to vialing, each material was brought to room temperature in its PFA container inside a laminar flow hood and gently mixed. In separate sessions, each solution was transferred with a manual pipette to sterile PFA vials, which were then capped and labeled. After the production run for that component was complete, all vials were placed into storage at 4 °C.

Material Qualification: Figure 1 displays electropherograms of the PCR amplification products at the Huntington's Disease locus for components A to F. Peaks for stutter products for one or more [CAG] repeat units less than the certified value ("reverse stutter") and one repeat more than the certified value ("forward stutter") are visible for alleles 15, 17, 29, 35, 36, 39, 40, and 45. There are two forward stutter peaks visible for the 50 allele and three for the 75 allele. Since genomic DNA rather than PCR products are supplied the stutter amounts may differ depending on user PCR conditions. PCR amplifications of the Huntington's Disease locus with more severe stutter than shown here can produce reverse stutter peaks that have greater height and area than the parent allele. The extent of both reverse and forward stutter increases with increasing number of [CAG] repeats.

Measurement of [CAG] Repeat Number: Both alleles for each component were sequenced in both forward and reverse directions or in multiple forward directions using Big Dye v 3.1 Sanger sequencing chemistry and capillary electrophoresis [6]. The number of [CAG] repeat units were counted. The counts were confirmed by genotyping the components and evaluating the relationships between the count and the apparent electrophoretic size of the PCR products and between the count and the peak area fraction of stutter products relative to the peak area of the parent allele.

Homogeneity Assessment: Material homogeneity was evaluated by comparison of relative peak areas of the PCR products at the Huntington's Disease locus. Five vials of each component were randomly selected and analyzed in duplicate.

Commutability of the Huntington's Disease CAG Repeat Certified Values: The commutability of the SRM 2393 components was evaluated by two independent European HD Registry reference laboratories [7].

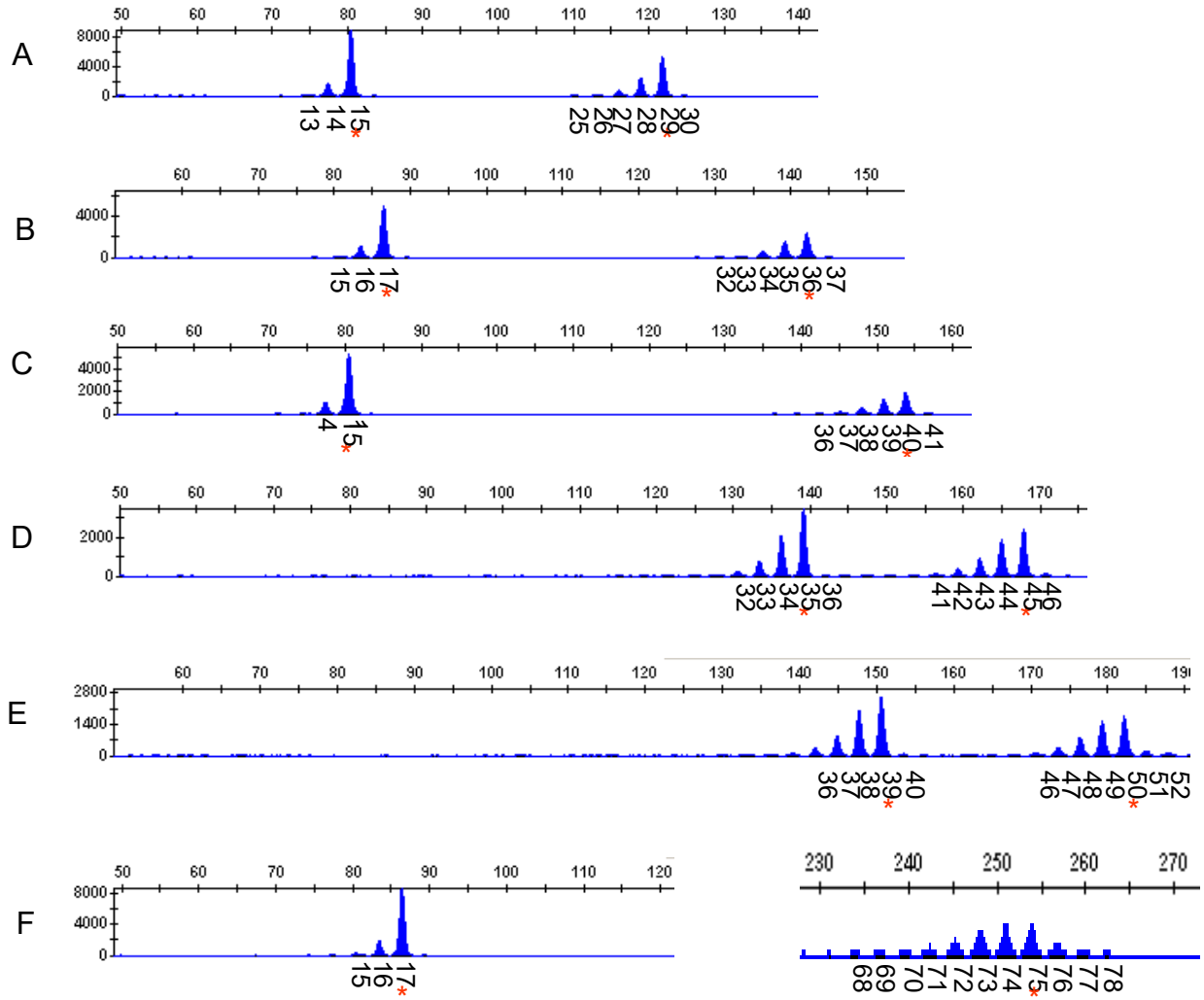


Figure 1. Example electropherograms of SRM 2393 components. The y-axis represents relative fluorescence units (RFUs). The RFU scale for the 75 repeat allele of Component F has been expanded to facilitate visualization of the "stutter" pattern and is not shown. The x-axis represents base pair size as estimated from an internal size standard. Values below the x-axis line are the number of [CAG] repeats for PCR products having peak heights of 50 RFU or more. The parent allele in each set of PCR amplification products is marked with a "*".

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APPENDIX B

Values of Potential Interest to Users: A NIST value of potential interest is considered to be a value that will be of interest and use to the SRM user, but insufficient information is available to assess adequately the uncertainty associated with the value or only a limited number of analyses were performed [4]. Values of potential interest to users cannot be used to establish metrological traceability. Values of potential interest are provided below for the DNA mass concentration of the SRM 2393 components. These values are the average of results from five quantitative polymerase chain reaction (PCR) methods calibrated with Component A from SRM 2372 Human DNA Quantitation Standard. Relatively high results from a commercial quantitative PCR method that amplifies at the hTERT locus were excluded from the average as this locus is sometimes over-expressed in cell line DNA [8,9].

Component	A	B	C	D	E	F
[DNA] ng/µL	8	10	12	9	10	9

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