National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2393

CAG Repeat Length Mutation in Huntington's Disease

This Standard Reference Material (SRM) is intended primarily 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. 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 of the Number of Huntington's Disease [CAG] Repeats: Table 1 lists the certified number of [CAG] repeats for each component. 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. Other techniques were explored and there is no reason to doubt these results. 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.

Information Values of Conventional DNA Mass Concentration: Table 2 lists information values 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 [4,5]. An information value is considered to be a value that will be of use to the SRM user, but insufficient information is available to assess the uncertainty associated with the value.

Expiration of Certification: The certification of **SRM 2393** is valid, within the measurement uncertainty specified, until **31 January 2016,** provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Warning and Instructions for Handling, Storage, and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Coordination of the technical measurements leading to the certification was under the direction of M.C. Kline and J.M. Butler of the NIST Biomolecular Measurement Division. Evaluation of the data was performed by D.L. Duewer of the NIST Chemical Sciences Division and reviewed by J.H. Yen of the NIST Statistical Engineering Division. M.C. Kline prepared the materials and performed the homogeneity and certification measurements with the assistance of staff from the divisions formerly know as the NIST Biochemical Science and Analytical Chemistry Divisions.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

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Certificate Revision History on Last Page

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WARNING AND INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

Handling: SRM 2393 IS A HUMAN-SOURCE MATERIAL. SINCE THERE IS NO CONSENSUS ON THE INFECTIOUS STATUS OF EXTRACTED DNA, HANDLE THE SRM 2393 COMPONENTS AS BIOSAFETY LEVEL 1 MATERIALS CAPABLE OF TRANSMITTING INFECTIOUS DISEASE [6]. SRM 2393 components and derived solutions should be disposed of in accordance with local, state, and federal regulations.

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. For the certified and informational values to be applicable, 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 and information values do not apply to any material remaining in recapped vials. **DO NOT EXPOSE ANY DNA SOLUTION TO DIRECT SUNLIGHT**.

PREPARATION AND ANALYSIS[(1)]

Sample Preparation: DNA extracts from the National Institute of General Medical Sciences (NIGMS) Human Genetic Cell Repository, Inherited Disorders subcollection [7] 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.

Table 1. Certified Values of the Number of Huntington's Disease [CAG] Repeats

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).

Table 2. Information Values of Conventional DNA Mass Concentration

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

[(1)]Certain commercial instruments, materials, or processes are identified in this report 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 instruments, materials, or processes identified are necessarily the best available for the purpose.

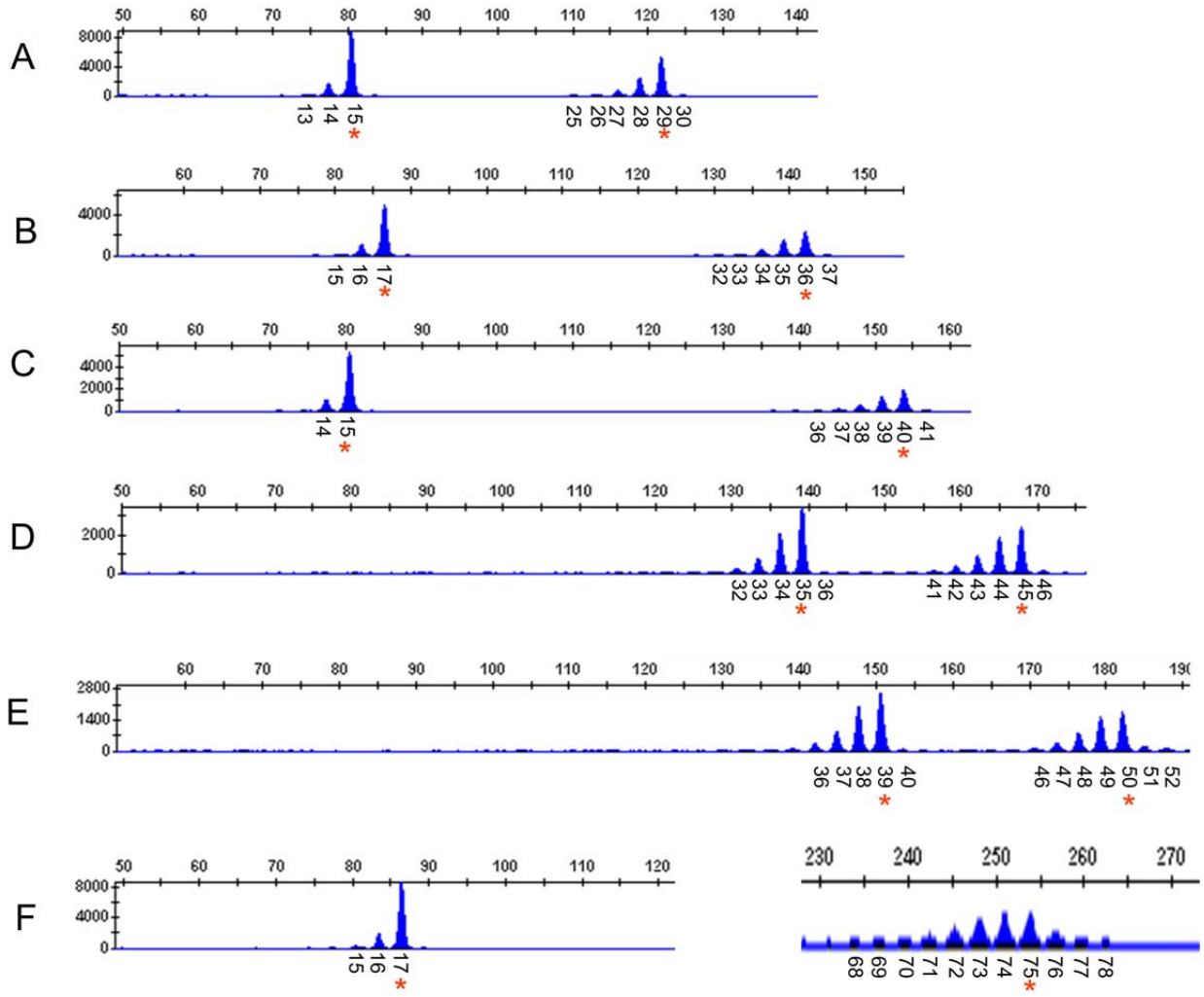

Figure 1. Example electropherograms of SRM 2393 components. The y-axis represents relative fluorescence units (RFUs). The RFU scale for the 75 repeat allele of Component F has been expanded to facilitate visualization of the "stutter" pattern and is not shown. The x-axis represents base pair size as 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 "*".

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 [8]. 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 [9].

REFERENCES

[1] Potter, N.T.; Spector, E.B.; Prior, T.W.; Technical Standards and Guidelines for Huntington Disease; *Standards and Guidelines for Clinical Genetics Laboratories*; American College of Medical Genetics: Bethesda, MD (2006); available at http://www.acmg.net/Pages/ACMG_Activities/stds-2002/HD.htm (accessed Dec 2012).

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[8] Kline, M.C.; Hill, C.R.; Decker, A.E.; Butler, J.M.; *STR Sequence Analysis for Characterizing Normal, Variant, and Null Alleles*; Forensic Sci. Int. Genet., electronic publication (2010); http://www.fsigenetics.com/article/S1872-4973(10)00156-0/abstract (accessed Dec 2012).

[9] European Huntington's Disease Network Registry; available at http://www.euro-hd.net/html/registry (accessed Dec 2012).

Certificate Revision History: 17 December 2012 (Reference laboratory information updated; editorial changes); 03 March 2011 (Original certificate issue date).

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