CERTIFICATION REPORT: The certification of the mass of lambda DNA in a solution
Certified Reference Material: ERM®-AD442k
Abstract:
This report describes the processing and certification of genomic lambda deoxyribonucleic acid (DNA) in a solution. ERM®-AD442k is certified for its lambda DNA mass, expressed in ng/µL. The DNA copy number concentration in cp/µL is provided as an indicative value. The material was produced according to ISO Guide 34:2009. A volume of approximately 400 mL of lambda DNA at an approximate concentration of 450 ng/µL was purchased from Promega Corporation and Benelux BV.
After homogenising and diluting this solution with TE buffer, 3100 vials of ERM-AD442k were produced. Each ERM-AD442k vial contains a certified DNA mass concentration of 57.53 ng/µL with an expanded combined uncertainty of 1.07 ng/µL. Using two different next generation sequencing (NGS) techniques (i.e. an Illumina platform and the GS Junior platform from Roche), the nucleic acid sequence of the lambda DNA in ERM-AD442k was verified. Non-lambda DNA sequences were identified in this material. The relative proportion of those sequences was estimated by NGS and further quantified by quantitative polymerase chain reaction (qPCR). Traces of the contaminating DNA, mainly coming from the Escherichia coli (E. coli) host used by Promega Corporation and Benelux BV to produce the lambda DNA, were negligible. Between-vial homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2006. Within-vial homogeneity was quantified to determine the minimum sample intake. The certified DNA mass concentration value was obtained by ultraviolet (UV) spectrophotometry and the indicative DNA copy number concentration value by digital PCR (dPCR). The DNA copy number concentration measured by dPCR is consistent with the DNA mass concentration determined by UV spectrophotometry. The measurements were performed according to the scope of accreditation to ISO/IEC 17025:2005. The material was characterised by an inter-laboratory comparison exercise performed by laboratories of demonstrated competence and with adherence to ISO/IEC 17025. Technically invalid results were removed; however no other outliers were eliminated on statistical grounds only. Uncertainties of the certified and indicative values were calculated in accordance with the Guide to the Expression of Uncertainty in Measurement (GUM) and include uncertainties relating to possible inhomogeneity and instability, and to characterisation. The material and its certified value are intended to be used for the calibration of DNA quantification methods, quality control and assessment of method performance. As any reference material, it can also be used to establish control charts or in validation studies. The indicative value of the material is, in contrast to the certified value, a value where the uncertainty was deemed too large to allow certification and is therefore less reliable than the certified value. The CRM is available in Axygen maximum recovery polypropylene vial containing a nominal volume of 1.1 mL lambda DNA in solution. The minimum amount of sample to be used is 50 µL for UV spectrophotometry and 68 µL for dPCR. The CRM was accepted as European Reference Material (ERM®) after peer evaluation by the partners of the European Reference Materials consortium.

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