

United States Geological Survey

Reston Stable Isotope Laboratory

Report of Stable Isotopic Composition

Reference Material KHS (Kudu Horn Standard)

(Hydrogen and Oxygen Isotopes in Kudu Horn keratin)

This reference material (RM) is intended for normalization of stable hydrogen ($\delta^2\text{H}$) and oxygen ($\delta^{18}\text{O}$) measurements of keratin in organic materials in wildlife and forensics research with a TC/EA (thermal conversion/elemental analyzer) and an isotope-ratio mass spectrometer when used with CBS (Caribou Hoof Standard) [1]. This RM consists of 0.5 g of KHS (Kudu Horn Standard) [1]. There is no limit on distribution. KHS was prepared by Dr. L. I. Wassenaar and Dr. K. Hobson [1] of Environment Canada, and this RM is distributed by the Reston Stable Isotope Laboratory (RSIL) of the U.S. Geological Survey, Reston, Virginia on their behalf.

Recommended values: Stable hydrogen and oxygen isotopic compositions are expressed herein as delta values [2] relative to VSMOW (Standard Mean Ocean Water) on scales normalized such that the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of SLAP (Standard Light Antarctic Precipitation) are -428‰ and -55.5‰ , respectively [3,4]. The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values below are preliminary and currently are being re-evaluated. The $\delta^2\text{H}$ values reported here are obtained by dual-water equilibration, followed by sample preparation using the glassy carbon reactor method. The $\delta^2\text{H}$ measurements using a chromium filled elemental analyzer as described by Gehre et al. [5] should be available in the near future. The mass fraction of total hydrogen and oxygen, and non-exchangeable fraction of hydrogen in KHS are preliminary.

Stable hydrogen isotopic composition (non-exchangeable fraction):	$\delta^2\text{H}_{\text{VSMOW-SLAP}} = -54.1 \pm 0.6 \text{‰}$	[1]
Mass fraction of total hydrogen:	$w_{\text{H}} = 6.5 \%$	
Mole fraction of exchangeable hydrogen:	$x_{\text{H-ex}} = 10.0 \pm 2.0 \%$	
Stable oxygen isotopic composition:	$\delta^{18}\text{O}_{\text{VSMOW-SLAP}} = +20.3 \pm 0.3 \text{‰}$	[1]
Mass fraction of oxygen:	$w_{\text{O}} = 22.5 \%$	

Technical coordination for this RM was provided by Haiping Qi of the RSIL.

Distribution and stability: KHS is stable at normal room temperatures. To minimize the potential for contamination, it is recommended that this RM be stored in the container in which it is supplied. The recommended reference values given in this Report of Stable Isotopic Composition may change after further evaluation. Users should contact the RSIL for updates to this report.

Instructions for use: Unknown keratin samples and keratin reference materials analyzed for both $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values typically need to be equilibrated with laboratory air at ambient temperature for at least 5 days. To minimize uncertainty caused by moisture that has not been removed, reference materials and unknowns need to be thoroughly dried prior to isotopic analysis based on experimental observations [6]. It is recommended that two keratin reference materials with different $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values be used for normalizing isotopic measurement results to the VSMOW-SLAP scale. In addition to KHS, other RMs could be CBS, USGS42 Tibetan human hair, and USGS43 Indian human hair. It is suggested that the mass of KHS, other references (such as CBS), and unknowns should be identical to minimize or cancel biases. It is also recommended that one should make the unknown sample powder size as close as possible to the powder size of KHS, recognizing that in some studies the sample cannot be pulverized.

Experimental studies indicate that improved measurement results can be achieved by analyzing samples for $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in separate runs because the operating conditions for $\delta^2\text{H}$ and $\delta^{18}\text{O}$ measurement with a TC/EA are different [5,6,7]. For $\delta^2\text{H}$ measurements, users should use a chromium filled elemental analyzer as described by Gehre et al. [5] because the elemental chromium will prevent production of hydrogen cyanide and will promote quantitative conversion of hydrogen to molecular hydrogen (H_2).

Reporting of stable-isotope-delta values: The following recommendations are provided for reporting of stable hydrogen and oxygen isotope-delta values [2]. It is recommended that:

- The $\delta^2\text{H}$ values of all hydrogen-bearing substances be expressed relative to VSMOW-SLAP on a scale where $\delta^2\text{H}_{\text{SLAP}2} = -427.5 \text{ ‰}$ or $\delta^2\text{H}_{\text{SLAP}} = -428 \text{ ‰}$ exactly [3,4,8].
- The $\delta^{18}\text{O}$ values of all oxygen-bearing substances be expressed relative to VSMOW-SLAP or relative to Vienna Peedee belemnite (VPDB; for carbonates) on a scale such that $\delta^{18}\text{O}_{\text{SLAP}2} = -55.5 \text{ ‰}$ or $\delta^{18}\text{O}_{\text{NBS}19} = -2.2 \text{ ‰}$, respectively.
- Authors should report δ values of internationally distributed (secondary) isotopic reference materials that were assumed for normalization of data for samples of similar chemical composition, as appropriate for the measurement method. In this manner, measurement results can be adjusted in the future as analytical methods improve and consensus values of internationally distributed isotopic reference materials change. Example text is:

“The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of keratin samples are reported relative to the VSMOW-SLAP scales, and on these scales the $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values of CBS and KHS are xxx, yyy, zzz, and uuu ‰, respectively [reference].”
- Reporting of δ values relative to SMOW and PDB (Peedee belemnite) be discontinued [9].

References

- [1] Wassenaar, L. I., Hobson, K. A., 2010, Two new keratin standards ($\delta^2\text{H}$, $\delta^{18}\text{O}$) for daily laboratory use in wildlife and forensic isotopic studies, poster, ISOECOL VII meeting, August 9–13, 2010: Fairbanks, Alaska, USA.
- [2] Coplen, T. B., 2011, Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results: *Rapid Communications in Mass Spectrometry*, v. 25, 2538–2560.
- [3] Gonfiantini, R., 1978, Standards for stable isotope measurements in natural compounds: *Nature*, v. 271, p. 534–536.
- [4] Coplen, T. B., 1994, Reporting of stable hydrogen, carbon, and oxygen isotopic abundances: *Pure and Applied Chemistry*, v. 66, p. 273–276.
- [5] Gehre, M., Renpenning, J., Gilevska, T., Qi, H., Coplen, T. B., Meijer, H. A. J., Brand, W. A., and Schimmelmann, A., 2015, On-line hydrogen-isotope measurements of organic samples using elemental chromium: An extension for high temperature elemental-analyzer techniques: *Analytical Chemistry*, v. 87, 5198–5205.
- [6] Qi, H.P., Coplen, T. B., 2011, Investigation of preparation techniques for $\delta^2\text{H}$ analysis of keratin materials and a proposed analytical protocol: *Rapid Communications in Mass Spectrometry*, v. 25, 2209–2222.
- [7] Qi, H.P., Coplen, T. B., Wassenaar, L. I., 2011, Improved online $\delta^{18}\text{O}$ measurements of nitrogen- and sulfur-bearing organic materials and a proposed analytical protocol: *Rapid Communications in Mass Spectrometry*, v. 25, 2049–2058.
- [8] International Atomic Energy Agency (IAEA), Reference Sheet for International Measurement Standards, http://nucleus.iaea.org/rpst/Documents/VSMOW2_SLAP2.pdf
- [9] Coplen, T. B., 1995, Discontinuance of SMOW and PDB: *Nature*, v. 375, 285.