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

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Reference Material 8461
Human Liver for Proteomics
REFERENCE MATERIAL INFORMATION SHEET

Purpose: This Reference Material (RM) is intended to support investigations of peptide and protein identification in complex samples such as those in mass spectrometry-based proteomics. RM 8461 can be used to help assess the confidence of peptide and protein identification within a laboratory or comparability between laboratories or among different measurement approaches. RM 8461 can also be used in the development and validation of new investigative approaches for identifying peptides and proteins.

Description: A unit of RM 8461 consists of a single vial containing approximately 0.5 g of freeze-dried human liver material.

Non-Certified Values: RM 8461 was produced to provide a complex human tissue sample for evaluating the performance of proteomics mass spectrometry instruments coupled to liquid chromatography (LC), software for the identification of proteins and peptides, and development of sample preparation methods [1]. Liver tissue was chosen to provide a "normalized" range of protein abundance and to provide sample complexity to cover the chromatographic "space". Non-certified values do not provide metrological traceability to the International System of Units (SI) or other higher-order reference system [2]. Non-certified qualitative values of identified proteins present in RM 8461 are listed in Table 1 as UniprotKB [3] accession numbers.

Period of validity: The non-certified values are valid until **31 March 2036**. 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. 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).

Marcela Najarro, Acting Chief
Chemical Sciences Division

Steven J. Choquette, Director
Office of Reference Materials

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Safety: THIS IS A HUMAN SOURCE MATERIAL THAT HAS BEEN STERILIZED BY GAMMA IRRADIATION AT A DOSE OF 60 kGy. Consult the Safety Data Sheet (SDS) exemption letter for hazard information.

Source: This RM was developed after an appropriate human subjects research determination by NIST.

Storage: RM 8461 should be stored at controlled room temperature (20 °C to 25 °C) and away from direct sunlight, until needed.

Use: RM 8461 IS INTENDED FOR LABORATORY USE ONLY. Prior to removal of a test portion for analysis, the contents should be mixed thoroughly. It is recommended that test portions for analysis equal to or greater than 1 mg are used.

Analysis: For qualitative measurands like protein identity, a conventional evaluation and expression of measurement uncertainty conforming to the JCGM GUM cannot be used. Table 1 lists the identities of 1619 proteins that have been established using identification algorithms whose reliability is generally recognized by experts in the field. The identified proteins in RM 8461 are inferred from bottom-up proteomics analysis using multiple search engines and multiple sample preparation methods. Commonly identified proteins were filtered from individual 1 mg preparations (n = 3) from eight jars of RM 8461 with replicate injections (n = 3). Proteins are included only when the following are met:

- false discovery rate (FDR) of less than 5 %,
- a spectral abundance of better than 20 % CV across a measurement set (including technical and process replicate analyses),
- having a minimum of three unique peptides identified from a stratified random sampling of eight jars across the entire production of RM 8461 and with Sequest HT, Mascot, MSAmanda, and Byonic algorithms.

NIST is confident of the identity of the non-certified qualitative values of 1619 proteins listed in Table 1 that met all criteria.

Non-Certified Values: Non-certified qualitative values of identified proteins present in RM 8461 are listed in Table 1 as UniprotKB [3] accession numbers. This table is also available as a download from the details page https://shop.nist.gov/ccrz__ProductDetails?sku=8461&cclcl=en_US. This consensus information is the best estimate based on currently available information as determined by bottom-up LC-MS/MS analyses and comparison of search engine algorithms; however, the evidence does not meet the NIST criteria for certification of identity.

Table 1. Non-Certified Values for Protein Content of RM 8461

Accession	Accession	Accession	Accession	Accession
A0A075B6S2	A0A0B4J1X5	A1L0T0	A3KMH1	A6ND91
A6NDB9	A6NLP5	E9PAV3	O00115	O00116
O00151	O00159-3	O00203	O00217	O00231-2
O00232	O00233	O00244	O00264	O00299
O00303	O00330	O00442-2	O00483	O00487
O00571	O00748	O00757	O00763	O14521
O14548	O14558	O14561	O14579	O14602
O14618	O14735	O14745	O14756	O14773
O14818	O14832	O14841	O14874	O14880
O14936	O14949	O14950	O14975	O14979
O14980	O15020	O15143	O15144	O15145
O15173-2	O15229	O15230	O15305	O15372
O15511	O43143	O43169	O43175	O43181
O43242	O43390	O43396	O43488	O43583
O43598	O43615	O43674	O43678	O43704
O43707	O43708	O43741	O43772	O43776
O43795	O43813	O43837	O43852-3	O43865
O43920	O60218	O60220	O60271	O60437
O60493	O60506	O60610	O60656	O60664
O60701	O60716	O60784	O60841	O60884
O75083	O75131	O75208	O75223	O75251
O75306	O75323	O75347	O75348	O75351
O75356	O75367-2	O75368	O75369	O75381

Accession	Accession	Accession	Accession	Accession
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O75608	O75795	O75822	O75832	O75874
O75880	O75884	O75891-3	O75915	O75936
O75947	O75955	O75964	O76021	O76054
O76062	O94760	O94788	O94826	O94874
O94875-12	O94903	O94905	O94929-3	O95140
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Q02252	Q02318	Q02338	Q02543	Q02790
Q02818	Q02878	Q02928	Q02978	Q03154
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Q13011	Q13057-2	Q13098-7	Q13131-2	Q13151
Q13155	Q13162	Q13200	Q13228-4	Q13242
Q13247	Q13263	Q13283	Q13310-3	Q13347
Q13363	Q13404-1	Q13409	Q13418	Q13423
Q13435	Q13442	Q13492	Q13496	Q13505
Q13546	Q13561-2	Q13576	Q13596	Q13630
Q13637	Q13642-4	Q13724	Q13838-2	Q13884
Q13885	Q13907-2	Q14019	Q14032	Q14103
Q14108	Q14117	Q14141	Q14152	Q14157-5
Q14165	Q14166	Q14204	Q14240-2	Q14247
Q14254	Q14258	Q14353	Q14376	Q14397
Q14444	Q14554	Q14558-2	Q14624	Q14651
Q14696	Q14697	Q14697-2	Q14749	Q14764
Q14847	Q14914	Q15005	Q15006	Q15019-2
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Q16822	Q16836-2	Q16851	Q16853	Q16891-2
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Q8NI22	Q8TAT6-2	Q8TC12	Q8TCJ2	Q8TEM1
Q8WUM4-2	Q8WUY1	Q8WVJ2	Q8WVM8	Q8WW59
Q8WWC4	Q8WWI1-3	Q8WWQ8	Q8WZA9	Q92499
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Q96AB3	Q96AE4-2	Q96AG4	Q96C11-3	Q96C19
Q96C23	Q96C86	Q96CM8-2	Q96CN7	Q96CW1
Q96DB5	Q96DC8	Q96DG6	Q96EK6	Q96EP5
Q96ER9	Q96EY1	Q96EY8	Q96F10	Q96FJ2
Q96FV2	Q96FW1	Q96G03	Q96G23	Q96GA7
Q96GD0	Q96GK7	Q96HC4	Q96HE7	Q96HJ9-2
Q96HR9-2	Q96HS1	Q96HY7	Q96I15	Q96I99
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Q96LJ7	Q96N76-2	Q96NU7	Q96P70	Q96PC5

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Q9BPW8	Q9BQE3	Q9BQE5	Q9BR76	Q9BRA2
Q9BRF8	Q9BRK5	Q9BRX8	Q9BS26	Q9BSE5
Q9BSH5	Q9BTV4	Q9BTZ2	Q9BUH6	Q9BUP0
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Q9BVK6	Q9BVL4	Q9BWD1	Q9BWS9	Q9BX66-11
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Q9BYV7	Q9H008	Q9H0N5	Q9H0W9	Q9H1K4
Q9H223	Q9H299	Q9H2A2	Q9H2F3	Q9H2M3
Q9H2U2-2	Q9H2X3-8	Q9H3G5	Q9H3K2	Q9H444
Q9H477	Q9H4A4	Q9H6R3	Q9H7Z7	Q9H845
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Q9NRX4	Q9NRZ7-3	Q9NSD9	Q9NSE4	Q9NTJ5
Q9NTK5	Q9NUI1	Q9NUJ1	Q9NUU7	Q9NVD7
Q9NVJ2	Q9NVS9	Q9NX40	Q9NXA8	Q9NXG2
Q9NYL4	Q9NYL9	Q9NYQ3-2	Q9NYU2	Q9NZ01
Q9NZ08-2	Q9NZ45	Q9NZL9	Q9NZN3	Q9NZN4
Q9NZT2	Q9P016	Q9P035	Q9P0J0-2	Q9P0L0-2
Q9P0Z9	Q9P258	Q9P2E9	Q9P2J5	Q9P2R3-4
Q9P2R7	Q9UBI6	Q9UBM7	Q9UBN7	Q9UBQ0
Q9UBQ5	Q9UBQ7	Q9UBR1	Q9UBR2	Q9UBS3
Q9UBS4	Q9UBX3-2	Q9UDR5	Q9UF12	Q9UFN0
Q9UGP8	Q9UHE5	Q9UHG3	Q9UHJ6	Q9UHK6-5
Q9UHQ9	Q9UI09	Q9UI17	Q9UI32	Q9UII2
Q9UIJ7	Q9UJ68	Q9UJ83	Q9UJM8	Q9UJS0-2
Q9UJZ1	Q9UKK9	Q9UKM9	Q9UKS6	Q9UKU7
Q9UKX2	Q9UL12	Q9UL25	Q9UL46	Q9ULA0
Q9ULC5-3	Q9ULD0	Q9ULV4-3	Q9UM00	Q9UMX0
Q9UN37	Q9UN86	Q9UNF0	Q9UNH7	Q9UNM6
Q9UNU6	Q9UNZ2-5	Q9UQ80	Q9Y224	Q9Y262
Q9Y281	Q9Y285	Q9Y2B0	Q9Y2H6	Q9Y2P5
Q9Y2Q3	Q9Y2R0	Q9Y2S2	Q9Y2T3-3	Q9Y2W1
Q9Y2X3	Q9Y315	Q9Y365	Q9Y376	Q9Y394
Q9Y3A5	Q9Y3A6	Q9Y3D2	Q9Y3D6	Q9Y3E5
Q9Y3F4-2	Q9Y3I0	Q9Y3Z3	Q9Y490	Q9Y4L1
Q9Y4P3	Q9Y4W6	Q9Y512	Q9Y5J7	Q9Y5J9
Q9Y5L4	Q9Y5M8	Q9Y5S9	Q9Y5X1	Q9Y5X3
Q9Y5Z4	Q9Y617	Q9Y619	Q9Y639	Q9Y646
Q9Y673	Q9Y678	Q9Y694	Q9Y696	Q9Y6B6
Q9Y6C2	Q9Y6C9	Q9Y6M9	Q9Y6N5	

Additional Information: The mass spectrometry proteomics raw data have been deposited to the ProteomeXchange Consortium [4] via the PRIDE [5] partner repository with the dataset identifier PXD013608 and Project DOI 10.6019/PXD013608.

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