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Product Information

MS Qual/Quant QC Mix

Product Number **MSQC1**
Storage Temperature –20 °C

Product Description

The MS Qual/Quant QC Mix is an injection-ready LC-MS sample designed to benchmark and monitor performance of both qualitative and quantitative proteomic platforms.

MS Qual/Quant QC Mix is intended for daily use to assess platform characteristics, including:

- Repeatability/Reproducibility between runs
- System stability (drift, chromatography, signal intensity, sensitivity, etc.)
- Inter- and intra-platform and lab comparisons

Qualitative Feature: The product consists of a pre-digested (trypsin) mixture of 6 human proteins, which span a 25-fold concentration range (see Table 1). The low-complexity, defined protein digest allows for benchmarking of MS^2 peptide sequencing. A FASTA file containing the sequence of the 6 human proteins is available for download on the product display page at www.sigmaaldrich.com.[1]

Quantitative Feature: Additionally, a set of 14 stable isotope labeled (SIL) peptides corresponding to 2–3 tryptic peptides for each protein has been incorporated into the protein digest. The SIL peptides span a concentration range greater than three orders of magnitude (see Table 1). The broad range in SIL peptide levels can be used to assess quickly detection sensitivity.

The 14 SIL peptides are spiked into the digested mixture over a broad theoretical range (from 50:1 to 0.2:1) of protein/SIL peptide ratios at each of the protein levels (see Table 1). This allows for benchmarking of quantitative ratio measurements by MRM or MS^1-based intensities.

Each 0.5 mL vial of MS Qual/Quant QC Mix contains 6 pre-digested (trypsin) human proteins plus 14 corresponding SIL peptides. The lyophilized mixture contains 7–8 μg of protein digest and SIL peptides with a small amount of Tris buffer salts (~1 μg), added to aid solubilization.

Lot-specific information regarding the light/heavy ratios empirically derived for each peptide can be found on the Certificate of Analysis. Instrument parameters used to generate the ratios provided on the Certificate of Analysis are provided in Table 2, and may be used as a guide for initial experiments. A Skyline[1] and Excel version of this table is available at www.sigmaaldrich.com for download. Users may choose to optimize their specific MRM parameters as necessary.

For initial evaluation, analysis of the equivalent of 1% of the vial contents is recommended. The amount required for subsequent and repeated analyses can be adjusted based on the specific performance metrics observed.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Daily Use: The supplied product has been purified by reverse phase chromatography (C18) to remove residual undigested protein or very hydrophobic peptides. Following reconstitution, the mixture is injection-ready without further processing.

Reconstitute the contents of the vial by adding 10–20 μl of an appropriate solvent directly to the vial followed by vigorous mixing. A recommended solvent is 0.1% formic acid with 20% acetonitrile.

Following reconstitution, the solubilized product can then be further diluted with 0.1% formic acid (as appropriate to reduce the % acetonitrile to be compatible with column loading) and dispensed as aliquots of desired volumes prior to use. Stability trials on the product prepared in this manner and maintained in 0.1% formic with 5% acetonitrile at 4 °C demonstrated little or no product degradation over a one week time period. Longer-term storage of aliquots should be at –20 °C.

Storage/Stability

The vial ships on wet ice and storage at –20 °C is recommended.

References

1. MacLean, B. *et al.*, *Bioinformatics*, **26**(7), 966-968 (2010).

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SG,FF,JLT,JW,HD,GCY,MAM 03/18-1

Table 1.

Protein Digest and Stable Isotope Labeled (SIL) peptide composition of MS Qual/Quant QC Mix.

Protein[1] (UniProt Accession Number)	Calc'd MW (Da)	Approximate protein per vial[2]		Corresponding SIL peptide sequences[3]	SIL Peptide Content per vial (pmol)[4]	Theoretical Ratio[5] Light : Heavy (Protein : SIL Peptide)
		pmol	µg			
Carbonic Anhydrase I (P00915)	28739	100	2.9	GGPFSDSY[R]	100	1
				VLDALQAI[K]	50	2
Carbonic Anhydrase II (P00918)	29115	100	2.9	AVQQPDGLAVLGIFL[K]	10	10
				SADFTNFDP[R]	2	50
NAD(P)H dehydrogenase (P15559)	30736	20	0.62	EGHLSPDIVAEQ[K]	20	1
				ALIVLAHSE[R]	10	2
C-reactive Protein (P02741)	23047	20	0.46	ESDTSYVSL[K]	2	10
				GYSIFSYAT[K]	0.4	50
Peptidyl-prolyl cis-trans isomerase A (P62937)	20176	4	0.08	FEDENFIL[K]	8	0.5
				VSFELFAD[K]	4	1
				TAENF[R]	2	2
Catalase (P04040)	59625	4	0.24	GAGAFGYFEVTHDIT[K]	20	0.2
				FSTVAGESGSADTV[R]	0.4	10
				NLSVEDAA[R]	0.08	50

[1] A FASTA file with all of the proteins sequences is available for download at the product display page at www.sigmaaldrich.com. Database search parameters should include carbamidomethylation of Cys (+57 Da).

[2] As estimated using standard colorimetric and UV protein assays prior to digestion with trypsin. The quantities of liberated protein derived peptides may differ due to digestion inefficiency and minor losses during C18 purification.

[3] Amino acid in [brackets] denotes site of label incorporation for heavy peptides as follows:

[K], $^{13}C_6{}^{15}N_2$
[R], $^{13}C_6{}^{15}N_4$

[4] As determined by amino acid analysis. SIL peptides are added to the digest following C18 purification and the SIL peptide quantities are considered to be more accurate than those of the protein-derived, light peptides. Detection sensitivity estimations should be based on SIL peptide quantity.

[5] Targeted ratio based on estimated protein derived and SIL peptide quantities (as determined above) prior to formulation, digestion, and purification. Lot-specific empirically determined ratios are provided in the Certificate of Analysis (available at the product page at www.sigmaaldrich.com and may differ from theoretical).

Table 2.
MRM transitions and instrument parameters used to determine Light/Heavy ratios reported on the Certificate of Analysis. Data was acquired using an API 4000 QTRAP mass spectrometer (ABSciex). A Skyline[1] and Excel version of this table is available for download at www.sigmaaldrich.com.

Protein (Uniprot Accession No.)	Peptide	MH+ (mono)	z (Q1)	Q1 m/z	Q3 m/z	Fragment ion type	CE	DP	CXP	EP
Carbonic Anhydrase I **(**P00915)	GGPFSDSYR	985.44	2	493.2	871.4	y7	21	80	12	10
			2	493.2	627.3	y5	27	80	12	10
			2	493.2	774.3	y6	33	80	12	10
	GGPFSDSY[R]	995.44	2	498.2	881.4	y7	21	80	12	10
			2	498.2	637.3	y5	27	80	12	10
			2	498.2	784.3	y6	33	80	12	10
	VLDALQAIK	970.59	2	485.8	643.4	y6	26	80	12	10
			2	485.8	758.4	y7	26	80	12	10
			2	485.8	871.5	y8	20	80	12	10
	VLDALQAI[K]	978.60	2	489.8	651.4	y6	26	80	12	10
			2	489.8	766.4	y7	26	80	12	10
			2	489.8	879.5	y8	20	80	12	10
Carbonic Anhydrase II **(**P00918)	AVQQPDGLAVLGIFLK	1668.97	2	835.0	1242.8	y12	36	80	12	10
			2	835.0	1030.7	y10	42	80	12	10
			2	835.0	973.6	y9	42	80	12	10
	AVQQPDGLAVLGIFL[K]	1676.97	2	839.0	1250.8	y12	36	80	12	10
			2	839.0	1038.7	y10	42	80	12	10
			2	839.0	981.6	y9	42	80	12	10
	SADFTNFDPR	1169.52	2	585.3	896.4	y7	25	80	12	10
			2	585.3	749.4	y6	25	80	12	10
			2	585.3	1011.5	y8	25	80	12	10
	SADFTNFDP[R]	1179.52	2	590.3	906.4	y7	25	80	12	10
			2	590.3	759.4	y6	25	80	12	10
			2	590.3	1021.5	y8	25	80	12	10
NAD(P)H dehydrogenase **(**P15559)	ALIVLAHSER	1108.65	2	554.8	712.4	y6	23	80	12	10
			2	554.8	811.4	y7	29	80	12	10
			2	554.8	924.5	y8	29	80	12	10
	ALIVLAHSE[R]	1118.66	2	559.8	722.4	y6	23	80	12	10
			2	559.8	821.4	y7	29	80	12	10
			2	559.8	934.5	y8	29	80	12	10
	EGHLSPDIVAEQK	1422.72	2	711.9	899.5	y8	30	80	12	10
			3	474.9	574.3	y5	36	80	12	10
			3	474.9	475.3	y4	36	80	12	10
	EGHLSPDIVAEQ[K]	1430.72	2	715.9	907.5	y8	30	80	12	10
			3	477.6	582.3	y5	36	80	12	10
			3	477.6	483.3	y4	36	80	12	10

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[1] MacLean, B. et al., Bioinformatics, **26**(7), 966-968 (2010).

Table 2 (continued)
MRM transitions and instrument parameters used to determine Light/Heavy ratios reported on the Certificate of Analysis. Data was acquired using an API 4000 QTRAP mass spectrometer (ABSciex). A Skyline[1] and Excel of this table is available for download at www.sigmaaldrich.com.

Protein (Uniprot Accession No.)	Peptide	MH+ (mono)	z (Q1)	Q1 m/z	Q3 m/z	Fragment ion type	CE	DP	CXP	EP
C-reactive Protein (P02741)	ESDTSYVSLK	1128.54	2	564.8	999.5	y9	36	80	12	10
			2	564.8	797.4	y7	24	80	12	10
			2	564.8	696.4	y6	30	80	12	10
	ESDTSYVSL[K]	1136.54	2	568.8	1007.5	y9	36	80	12	10
			2	568.8	805.4	y7	24	80	12	10
			2	568.8	704.4	y6	30	80	12	10
	GYSIFSYATK	1136.56	2	568.8	829.5	y7	30	80	12	10
			2	568.8	916.5	y8	30	80	12	10
	GYSIFSYAT[K]	1144.56	2	572.8	837.5	y7	30	80	12	10
			2	572.8	924.5	y8	30	80	12	10
Peptidyl-Prolyl cis-trans isomerase A (P62937)	FEDENFILK	1154.57	2	577.8	878.5	y7	24	80	12	10
			2	577.8	634.4	y5	30	80	12	10
	FEDENFIL[K]	1162.57	2	581.8	886.5	y7	24	80	12	10
			2	581.8	642.4	y5	30	80	12	10
	VSFELFADK	1055.54	2	528.3	593.3	y5	22	80	12	10
			2	528.3	722.4	y6	22	80	12	10
			2	528.3	869.4	y7	28	80	12	10
	VSFELFAD[K]	1063.54	2	532.3	601.3	y5	22	80	12	10
			2	532.3	730.4	y6	22	80	12	10
			2	532.3	877.4	y7	28	80	12	10
	TAENFR	737.36	2	369.2	565.3	y4	21	70	12	10
			2	369.2	636.3	y5	21	70	12	10
	TAENF[R]	747.36	2	374.2	575.3	y4	21	70	12	10
			2	374.2	646.3	y5	21	70	12	10
Catalase (P04040)	FSTVAGESGSADTVR	1483.70	2	742.4	792.4	y8	38	80	12	10
			2	742.4	921.4	y9	38	80	12	10
			2	742.4	978.5	y10	32	80	12	10
	FSTVAGESGSADTV[R]	1493.70	2	747.4	802.4	y8	38	80	12	10
			2	747.4	931.4	y9	38	80	12	10
			2	747.4	988.5	y10	32	80	12	10
	NLSVEDAAR	974.49	2	487.8	660.3	y6	26	80	12	10
			2	487.8	747.4	y7	20	80	12	10
			2	487.8	561.3	y5	20	80	12	10
	NLSVEDAA[R]	984.49	2	492.8	670.3	y6	26	80	12	10
			2	492.8	757.4	y7	20	80	12	10
			2	492.8	571.3	y5	20	80	12	10
	GAGAFGYFEVTHDITK	1712.83	2	856.9	1089.6	y9	43	80	12	10
			2	856.9	1252.6	y10	43	80	12	10
			2	856.9	1309.6	y11	43	80	12	10
	GAGAFGYFEVTHDIT[K]	1720.83	2	860.9	1097.6	y9	43	80	12	10
			2	860.9	1260.6	y10	43	80	12	10
			2	860.9	1317.6	y11	43	80	12	10

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[1] MacLean, B. et al., Bioinformatics, **26**(7), 966-968 (2010).