

# ABRF-sPRG09: Development of a Quantitative Proteomics Standard

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## Introduction

The Proteomics Standards Research Group (sPRG) initiated a study in 2007 that focused on development of a mixture of standard proteins that contained appropriate stable isotope labeled (SIL) peptides and could be used as a model for quantitative plasma proteomics. A set of 350 human plasma proteins was evaluated extensively, with the goal of selecting 50 proteins that would be distributed over five orders of magnitude in concentration. After lengthy consideration of the project, it was decided that there would be too many challenges associated with analysis of this type of sample. Efforts have now focused on development of a simplified standard that is based on human plasma proteins and would be suitable for use in assessing a laboratory's capabilities for absolute quantitative analysis.

Drawing from the information gained previously from evaluation of the 350 human plasma proteins, 5 candidate proteins were selected for this study. Sigma Life Science (St. Louis, MO) graciously agreed to partner with the sPRG in this project and prepared all protein samples, digests and stable isotope labeled (SIL) peptides for subsequent analyses.

## MATERIALS and METHODS

### Protein Preparation

Proteins were purified using either a 2-80% ACN/0.1% TFA gradient over 26 minutes or 20-80% ACN/0.1% TFA gradient over 20 minutes using a 25 cm x 4.6 mm C<sub>8</sub> BioVidePore HPLC Column (Supelco, Bellefonte, PA) coupled to a Shimadzu Prominence HPLC. The fractions corresponding to the protein were divided into several aliquots for Amino Acid Analysis, pilot scale samples, and study sample formulation, and stored at -80°C until use.

### Peptide Preparation

SIL peptides (Sigma-Genosys, The Woodlands, TX) were synthesized using standard solid-phase peptide synthesis techniques, purified by RP-HPLC, quantified by Amino Acid Analysis and verified by LC-MS for purity and mass. SIL peptide stocks were diluted 10 fold in 20% ACN/0.1% TFA and the stock SIL solution was formulated using 120 pmol of each SIL peptide (1 pmol of each SIL per sample), diluted to 400 µL with 20% ACN/0.1% TFA and stored at -80°C until further use.

All (SIL) peptides were spiked in at 1 pmol with adjustments to be made to protein amounts so that the Protein:SIL peptide ratios were as follows:

UBIQ\_HUMAN- 10 pmol protein: 1 pmol SIL (10:1)

SYHC\_HUMAN- 5 pmol protein: 1 pmol SIL (5:1)

ALBU\_HUMAN- 1 pmol protein: 1 pmol SIL (1:1)

NQO2\_HUMAN 1 pmol protein: 1 pmol SIL (1:1)

PRDX1\_HUMAN- 0.1 pmol protein: 1 pmol SIL (0.1:1)

### Sample Preparation

Before digestion, the proteins were mixed at the above relative amounts based on AAA, and lyophilized overnight with 10 mM NH<sub>4</sub>HCO<sub>3</sub>, pH 8.0. A single sample was digested which included enough protein for approximately 120 samples (60 µg total protein). The sample was suspended in 80 µL of 50 mM NH<sub>4</sub>HCO<sub>3</sub>, 2 mM TCEP, 1 mM CaCl<sub>2</sub>, pH 8.6, and warmed at 60°C for 15 minutes. The proteins were alkylated with iodoacetamide at a final concentration of 5 mM and quenched with 4 mM TCEP. The sample was diluted to 200 µL and pH adjusted with HCl to pH 8.0 prior to the addition of trypsin. Trypsin was reconstituted in HPLC grade H<sub>2</sub>O (125 ng/µL) and 2 µg was added to the protein solution. The digestion was allowed to proceed for 16 hours at 37°C. A second aliquot of trypsin was added after 16 hours and digestion occurred for another 6 hours at 37 °C. The digestion was stopped with 1% formic acid, diluted to 800 µL with 50 mM NH<sub>4</sub>HCO<sub>3</sub>, pH 8.0, and stored at -80°C until further use.

### Formulation and Distribution

Pilot samples were analyzed by the sPRG and determined to be valid; the remainder of the frozen digest was spiked with the SIL stock, dispensed (10 µL/ sample) and dried by vacuum centrifugation to completion. Two random samples were removed for final analysis and two additional random samples were removed and incubated in a 37°C water bath for 48 hours in order to determine if the samples would be stable for room temperature shipment.

### Distribution

The sample was distributed to requesters (54) who were advised to reconstitute it in a buffer suitable for MS analysis (i.e., 0.1% Formic Acid/ H<sub>2</sub>O) using vortexing and sonication for 5 minutes.

Results were submitted anonymously through an on-line survey and included experimental, instrumental and other details.

5-DIGIT Identifier	ionization	Mass Analyzer	Peptides Quantified	Median % Consensus	Median Absolute Deviation	ALBU_HUMAN LVNEVTEFAK SLHTLFGDK AEFAEVS	NQO2_HUMAN NVAVDLSR	PRDX1_HUMAN DISLSDYK ADEGISFR GLFIIDDK LVQAFQFTDK	SYHC_HUMAN AALEELVK DOGGELLSR GLAPEVADR IFSIVEOR	UBIQ_HUMAN TITLVEPDSIENVK TSLSDYNIK ESTLHLVLR	Proteins Quantified	Median % of Consensus	Median Absolute Deviation	ALBU_HUMAN mean con dev	NQO2_HUMAN mean con dev	PRDX1_HUMAN mean con dev	SYHC_HUMAN mean con dev	UBIQ_HUMAN mean con dev																
RG01	Electrospray	LIT-FT	15	91.71%	6.71%	89.20%	94.53%	91.71%	85.70%	85.00%	78.68%	107.00%	82.76%	100.00%	78.74%	92.07%	92.92%	110.85%	79.58%	96.81%	5	92.10%	3.87%	0.95	93.86%	0.80	85.70%	0.09	88.15%	3.53	92.10%	8.58	95.96%	
RG02	Electrospray	LIT-FT	15	91.46%	6.99%	97.00%	84.91%	99.51%	97.85%	110.00%	100.64%	80.00%	103.45%	86.89%	107.43%	78.30%	96.46%	92.83%	91.73%	92.83%	81.87%	5	94.78%	1.88%	0.96	95.05%	0.91	97.85%	0.10	94.78%	3.56	92.90%	7.87	87.89%
RG03	Electrospray	LIT-FT	15	100.49%	13.53%	92.00%	90.57%	100.49%	91.40%	90.00%	82.34%	90.00%	68.97%	114.51%	130.86%	107.23%	115.57%	130.68%	140.20%	114.02%	5	96.04%	10.74%	0.97	96.04%	0.85	91.40%	0.09	85.30%	4.51	117.70%	11.32	126.55%	
RG04a	Electrospray	LIT-Orbi	15	94.83%	11.66%	75.00%	100.94%	101.46%	95.70%	60.00%	91.34%	100.00%	94.83%	108.34%	73.40%	106.48%	84.20%	113.65%	59.28%	82.43%	5	94.21%	1.49%	0.96	95.05%	0.89	95.70%	0.09	85.30%	3.61	94.21%	7.62	85.19%	
RG04b	Electrospray	LIT-Orbi	14	103.56%	6.68%	106.00%	100.94%	213.66%	98.92%	-	82.34%	90.00%	94.83%	105.23%	105.12%	118.69%	5	116.44%	0.92	98.92%	0.10	94.78%	11.64%	0.97	98.92%	0.37	114.04%	9.89	110.56%					
RG04c	Electrospray	LIT-Orbi	14	105.25%	3.78%	106.00%	100.94%	108.29%	105.38%	-	82.34%	90.00%	94.83%	151.25%	102.00%	93.77%	106.37%	105.92%	105.12%	118.69%	5	106.93%	3.63%	1.08	106.93%	0.88	105.38%	0.10	94.78%	4.37	114.04%	9.89	110.56%	
RG04d	Electrospray	LIT-Orbi	15	92.32%	5.29%	94.00%	100.94%	99.51%	88.17%	80.00%	91.49%	100.00%	94.83%	92.25%	73.14%	87.03%	84.20%	97.71%	92.32%	93.55%	5	94.35%	5.65%	1.01	100.93%	0.92	88.17%	0.10	94.78%	3.27	85.34%	8.44	94.25%	
RG04e	Electrospray	LIT-Orbi	15	100.00%	10.28%	108.00%	100.00%	99.51%	84.95%	80.00%	64.04%	80.00%	103.45%	103.78%	138.57%	105.24%	166.51%	102.97%	109.88%	89.72%	5	92.12%	7.11%	1.04	95.05%	0.39	94.95%	0.09	85.30%	5.00	130.49%	8.24	92.12%	
RG04f	Electrospray	LIT-Orbi	15	97.00%	3.53%	97.00%	100.00%	97.56%	81.72%	100.00%	100.64%	100.00%	103.45%	95.20%	98.00%	88.03%	85.85%	65.94%	93.47%	93.27%	5	92.38%	7.64%	1.01	100.00%	0.76	81.72%	0.11	104.26%	3.54	92.38%	7.58	84.74%	
RG05	Electrospray	LIT-Orbi	15	88.78%	18.78%	86.00%	59.43%	88.78%	100.00%	70.00%	54.89%	50.00%	25.86%	170.53%	78.22%	110.00%	5	96.30%	0.23	100.00%	0.03	28.43%	18.08%	0.39	100.00%	0.73	96.30%	3.69	96.30%	11.45	128.00%			
RG06	Electrospray	QqQ	15	113.00%	6.73%	105.00%	90.57%	99.51%	82.80%	80.00%	82.34%	110.00%	103.45%	94.40%	99.71%	104.99%	102.59%	102.05%	112.16%	101.12%	5	101.52%	2.90%	1.04	102.97%	0.77	82.80%	0.10	94.78%	3.89	101.52%	9.34	104.42%	
RG07	Electrospray	QqQ	14	99.41%	3.02%	95.00%	107.55%	92.68%	100.00%	100.00%	100.64%	110.00%	94.83%	91.45%	98.00%	-	98.82%	97.95%	102.05%	102.80%	5	100.00%	0.95%	1.01	100.00%	0.93	100.00%	0.11	104.26%	3.67	95.77%	9.03	100.95%	
15006	MALDI	TOF/TOF	11	95.01%	6.56%	86.12%	-	-	113.10%	-	187.10%	-	117.41%	96.54%	95.01%	100.13%	88.45%	93.49%	89.89%	87.89%	5	95.85%	10.58%	0.86	85.27%	1.05	113.10%	0.15	144.54%	3.67	95.85%	8.06	90.05%	
19567	MALDI	TOF/TOF	15	101.93%	9.29%	109.28%	90.95%	101.93%	107.00%	100.00%	115.90%	126.55%	106.08%	89.10%	92.64%	105.55%	106.08%	93.47%	92.47%	92.83%	5	95.46%	3.67%	1.04	103.46%	1.00	107.00%	0.13	120.66%	3.82	99.79%	7.97	89.15%	
24103	MALDI	TOF/TOF	13	109.70%	20.01%	109.70%	99.81%	87.90%	107.63%	-	75.94%	-	51.72%	129.71%	115.17%	117.96%	104.62%	148.94%	146.36%	142.54%	5	107.63%	10.01%	1.02	100.89%	1.00	107.63%	0.05	45.49%	4.51	117.64%	13.00	145.32%	
46011	MALDI	TOF/TOF	12	98.38%	7.92%	113.00%	102.69%	-	-	91.49%	40.00%	120.69%	92.74%	-	-	-	-	107.00%	102.69%	105.61%	4	95.79%	5.63%	1.10	108.91%	-	-	0.09	85.30%	3.70	96.56%	8.50	95.03%	
47886	MALDI	TOF/TOF	14	108.96%	7.09%	117.00%	100.00%	104.39%	111.83%	210.00%	192.13%	-	112.07%	65.16%	108.57%	84.79%	104.48%	115.10%	88.09%	109.35%	5	111.35%	2.44%	1.10	108.91%	1.04	111.83%	0.17	161.13%	3.53	92.12%	9.96	111.35%	
72972	MALDI	TOF/TOF	15	105.37%	19.19%	94.00%	100.00%	105.37%	90.32%	660.00%	155.54%	340.00%	60.34%	69.99%	112.00%	98.25%	110.38%	184.39%	86.17%	145.23%	5	101.98%	11.66%	1.03	101.98%	0.84	90.32%	0.31	293.82%	3.79	98.91%	12.51	139.85%	
72973	MALDI	TOF/TOF	15	100.48%	40.49%	96.00%	8.49%	100.48%	90.32%	60.00%	45.75%	60.00%	51.72%	115.58%	135.71%	113.42%	116.51%	409.42%	142.51%	177.38%	5	99.01%	27.30%	1.00	99.01%	0.05	47.39%	4.84	126.31%	15.05	168.25%			
26402	Electrospray	3D IT	15	94.63%	10.38%	99.00%	91.51%	94.63%	105.38%	160.00%	128.09%	130.00%	120.69%	95.47%	86.29%	88.53%	88.21%	76.57%	84.25%	67.29%	5	96.04%	9.34%	0.97	96.04%	0.98	105.38%	0.14	132.69%	3.47	90.56%	6.71	75.01%	
92329	Electrospray	3D IT	4	103.16%	11.64%	95.00%	114.32%	73.17%	118.28%	-	-	-	-	-	-	-	-	-	-	-	2	106.66%	11.62%	0.96	95.05%	1.10	118.28%	-	-	-	-	-		
13541b	Electrospray	LIT	15	107.80%	43.18%	102.00%	166.98%	46.83%	7276.34%	560.00%	247.03%	210.00%	146.55%	107.80%	106.86%	97.51%	64.62%	47.22%	67.22%	109.35%	5	100.47%	16.40%	0.89	88.12%	6.67	7276.34%	0.24	227.47%	3.85	100.47%	7.52	84.07%	
26019	Electrospray	LIT	15	101.46%	4.25%	98.00%	100.94%	101.46%	72.04%	120.00%	100.64%	110.00%	103.45%	101.10%	105.71%	109.48%	105.90%	103.10%	97.31%	92.52%	5	101.98%	2.28%	1.03	101.98%	0.67	72.04%	0.11	104.26%	4.09	106.74%	9.04	101.06%	
29850	Electrospray	LIT	14	82.83%	39.90%	92.48%	132.80%	-	79.86%	29.32%	73.03%	9.52%	79.94%	160.90%	85.71%	124.69%	141.51%	120.77%	76.82%	18.69%	5	79.86%	19.15%	1.00	99.01%	0.74	79.86%	0.05	50.09%	5.00	130.48%	6.00	67.08%	
30030	Electrospray	LIT	15	102.44%	7.65%	102.00%	122.84%	105.37%	103.00%	102.44%	108.79%	130.00%	93.10%	102.66%	102.66%	94.75%	96.26%	93.10%	102.66%	94.75%	5	104.44%	0.96	1.06	104.95%	0.96	113.74%	0.12	113.74%	3.50	101.34%	8.70	97.26%	
92125	Electrospray	LIT	15	108.34%	4.23%	110.00%	103.77%	99.51%	108.60%	100.00%	118.94%	110.00%	103.45%	108.34%	112.57%	104.49%	110.61%	108.34%	113.32%	105.98%	5	108.60%	1.67%	1.08	106.93%	1.01	108.60%	0.12	113.74%	4.22	110.13%	9.50	106.20%	
118542	Electrospray	LIT-FT	12	100.00%	4.16%	100.00%	94.34%	97.56%	86.02%	100.00%	91.49%	100.00%	86.21%	-	-	-	-	102.66%	1															