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SILAC Hi3 Standards

CONTENTS

I. INTRODUCTION

The Hi3 stable isotope labeled (SIL) standards (Phos B and ClpB) provides an isotopically unique exogenous standard to perform Hi3 relative protein quantitation.

- Minimize interferences with the endogenous peptides
- Perform relative protein quantitation of mixed proteome samples
- Add a second level of internal quality control (*i.e.*, spike Hi3 SILAC standards toward the lower limit of detection).

These standards, along with their unlabeled counterparts, are quantitated via AAA to equimolar ratios to provide greater accuracy to each analysis. They are greater than 95% pure and have been verified by mass identification. In addition, each vial is in a Waters Maximum Recovery Vial which simplifies the sample preparation process.

These standards are primarily intended for use with the Hi3 quantification method for MS^E proteomics data processed with ProteinLynx GLOBAL SERVER.™ The *E. coli* standard is intended for samples of animal origin, and the Phos B standard is intended for samples of microbial origin. The standards may also be of use in the evaluation and benchmarking of proteomic LC/MS systems comprised of nanoACQUITY UPLC® and SYNAPT® and Xevo® time-of-flight mass spectrometers.

Table 1. SILAC Hi3 Phos B Standard

Peptide	Sequence	Average Molecular Weight	Concentration
SILAC Phos B Sequence 1	H2N-VLYPNDNFFEGK-OH	1449.7066	1 nmol/vial
SILAC Phos B Sequence 2	H2N-TC*AYTNHTVLPEALER-OH	1883.9227	1 nmol/vial
SILAC Phos B Sequence 3	H2N-IGEEYISDLDQLRK-OH	1685.8762	1 nmol/vial
SILAC Phos B Sequence 4	H2B-LLSYVDDEAFIR-OH	1449.7531	1 nmol/vial
SILAC Phos B Sequence 5	H2N-LITAIGDVVNHDPVVGDR-OH	1899.0242	1 nmol/vial
SILAC Phos B Sequence 1	H2N-VLYPNDNFFEGK-OH	1269.6055	1 nmol/vial

The cysteine in sequence 2 is carbamidomethylated.

Table 2. SILAC Hi3 *E. coli* Standard

Peptide	Sequence	Average Molecular Weight	Concentration
SILAC *E. coli* ClpB Sequence 1	H2N-VIGQNEAVDAVSNAIR-OH	1664.8873	1 nmol/vial
SILAC *E. coli* ClpB Sequence 2	H2N-NNPVLIGEPGVGK-OH	1300.7276	1 nmol/vial
SILAC *E. coli* ClpB Sequence 3	H2N-AIDLIDEAASSIR-OH	1382.7433	1 nmol/vial
SILAC *E. coli* ClpB Sequence 4	H2N-VTDAEIAEVLAR-OH	1295.7113	1 nmol/vial
SILAC *E. coli* ClpB Sequence 5	H2N-AIQQQIENPLAQQILSGELVPGK-OH	2482.3751	1 nmol/vial
SILAC *E. coli* ClpB Sequence 1	H2N-LPQVEGTGGDVQPSQDLVR-OH	2004.0304	1 nmol/vial

II. STORAGE AND STABILITY

Lyophilized peptides generally have excellent stabilities, often showing little or no degradation after a few years at -20 °C. Long term storage (>1 year) should be at -80 °C desiccated, medium term storage (1–12 months) should be at -20 °C desiccated, short term storage (<1 month) may be at 4 °C. Once reconstituted the solution should be used immediately to avoid degradation of peptides which would compromise the quantitative benefit.

III. RECONSTITUTION OF THE SILAC HI3 DIGESTION STANDARDS

The SILAC Hi3 Standards are originally in a concentration of 1 nmol/vial of either the ClpB_ECOLI or the PYGM_RABIT. The lyophilized peptides were re-suspended in 1000 µL of 3% acetonitrile w/ 0.1% Trifluoroacetic acid. The resulting concentration was 1 pmol/µL. The SILAC Hi3 standards were then diluted to 25 fmol/µL, *i.e.*, 25 µL of each solution was diluted to 1000 µL. Because peptides can adhere to glass and other surfaces resulting in significant losses from highly dilute solutions, further dilutions should be made just prior to addition to samples and excess diluted standard should be discarded. Unused stock solutions may be stored in a freezer for a brief time. Analysis of small amounts of standard (ca. 50 fmol) should only be carried out in the presence of a sample matrix such as *E. coli* digest of a serum digest (60 ng/µL or greater).

IV. USING THE SILAC HI3 DIGESTION STANDARDS FOR QUANTIFICATION

The following is an example of the standards run on a Waters SYNAPT G2-S HDMS mass spectrometer and the corresponding results.

Conditions:

Solvent A: 0.1% formic acid in water

Solvent B: 0.1% formic acid in acetonitrile

Weak wash: 3% ACN w/ 0.1% TFA

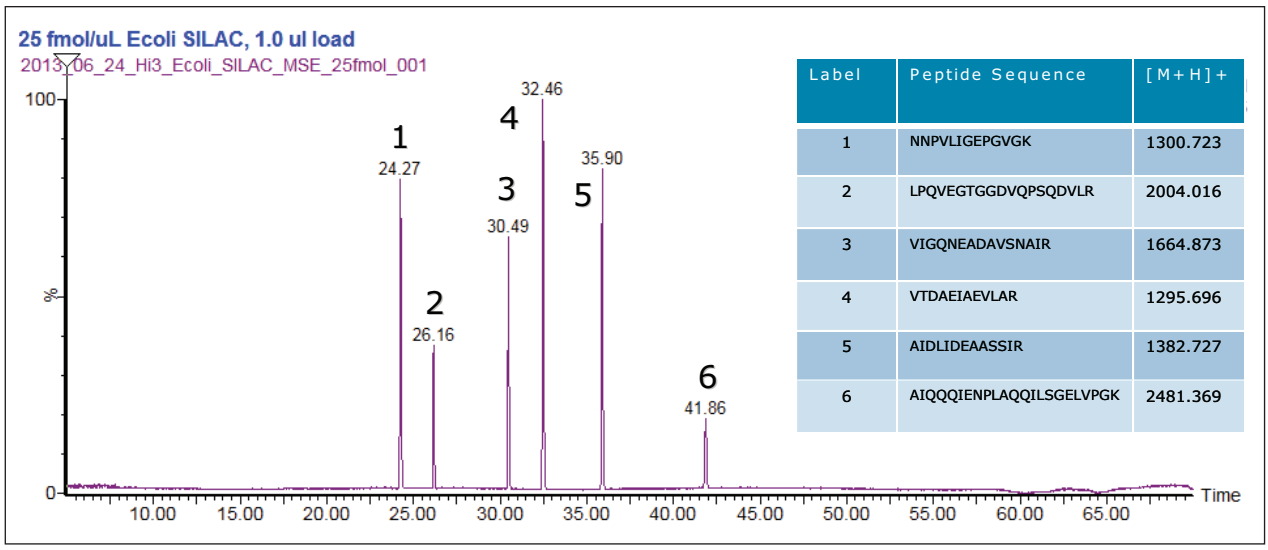
Trapping: 10 µL/min for 3 min, 10 µL loop

Trap: ACQUITY UPLC® PST C_{18} nanoACQUITY® Trap, 5 µm, 180 µm x 20 mm

Gradient Profile:

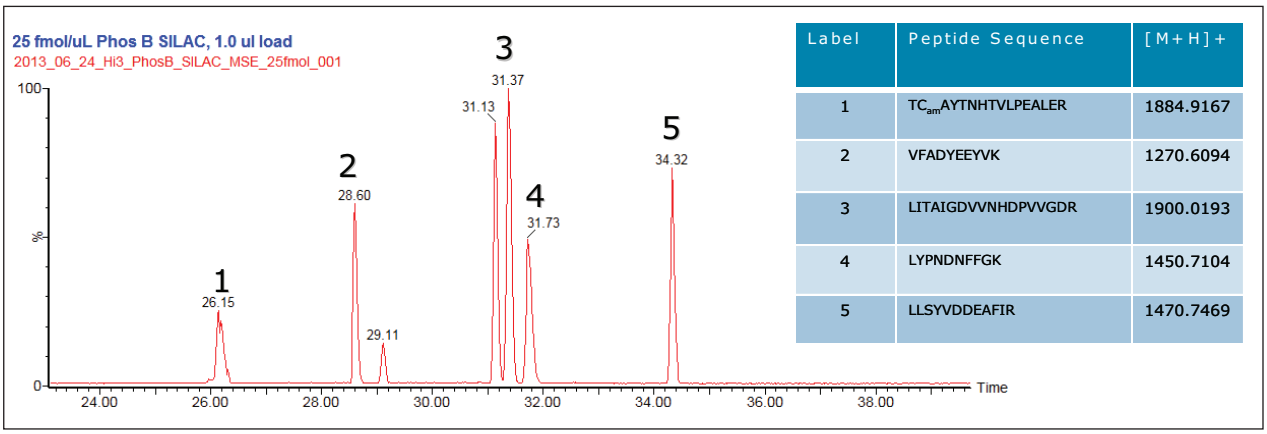
Time	Flow Rate (µL/min)	%A	%B	Curve
Initial	0.500	95.0	5.0	6
54.00	0.500	60.0	40.0	6
55.80	0.500	15.0	85.0	6
59.40	0.500	15.0	85.0	6
61.20	0.500	95.0	5.0	6
72.00	0.500	95.0	5.0	6

Table 3. Base Peak Intensity Chromatograms: 1D MS^E – 25 fmol SILAC Hi3 ClpB E. coli.



Label	Peptide Sequence	[M+H]+
1	NNPVLIGEPGVGK	1300.723
2	LPQVEGTGGDVQPSQDVLR	2004.016
3	VIGQNEADAVSNAIR	1664.873
4	VTDAEIAEVLAR	1295.696
5	AIDLIDEAASSIR	1382.727
6	AIQQQIENPLAQQILSGELVPGK	2481.369

Figure 1. Base Peak Intensity Chromatograms: 1D MS^E – 25 fmol Hi3 SILAC ClpB E. coli.



Label	Peptide Sequence	[M+H]+
1	TC_{am}AYTNHTVLPEALER	1884.9167
2	VFADYEEYVK	1270.6094
3	LITAIGDVVNHDPVVGDR	1900.0193
4	LYPNDNFFGK	1450.7104
5	LLSYVDDEAFIR	1470.7469

Figure 2. Base Peak Intensity Chromatograms: 1D MS^E – 25 fmol Hi3 SILAC PhosB.

V. ORDERING INFORMATION

Product Description	Qty	Part Number
SILAC Hi3 Phos B Standard	1/pk	186007083
SILAC Hi3 *E. coli* Standard	1/pk	186007084
Hi3 Phos B Standard	1/pk	186006011
Hi3 *E. coli* Standard	1/pk	186006012
MassPREP™ Digestion Standard, Phosphorylase B	1/pk	186002326
MassPREP *E. coli* Digestion Standard	1/pk	186003196
ACQUITY UPLC PST C_{18} nanoACQUITY Trap, 5 µm, 180 µm x 20 mm		186006527
LCGC Certified Clear Glass, 12 x 32 mm, Screw Neck, Max Recovery Vial	100/pk	186000327C

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