

SOP_001_NU_1_2_Top_Down_Standard_v2E_LF_CJD

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❖ Reagent and Materials List

Item	Part Number	Vendor
Pierce Intact Protein Standard Mix	A35526	Thermo Fisher Scientific
Optima Grade Water	W6-4	Fisher Scientific
Optima Grade Acetonitrile	A955-4	Fisher Scientific
MS-Grade Formic Acid	PI-28905	Fisher Scientific
1.5 mL Protein LoBind Microcentrifuge Tubes	13-698-794	Fisher Scientific

❖ Standard Components (from Pierce Intact Protein Standard Product Data Sheet)

Protein Name	Organism	Theoretical monoisotopic mass (Da)
Protein G	<i>S. dysgalactiae</i>	21429.75915
Protein AG (chimeric)	<i>S. dysgalactiae</i>	50429.84641
IGF-I LR3	<i>H. sapiens</i>	9105.34872
Thioredoxin	<i>H. sapiens</i>	11858.04393
Carbonic Anhydrase II	<i>B. taurus</i>	28963.6881
Exo Klenow	<i>E. coli</i>	67959.42515

❖ Preparation

- ◆ Dilute one 76 µg vial into 152 µL of Optima H₂O, for a final concentration of 0.5 µg/µL.
- ◆ Divide final mixture into 2 µL aliquots (in LoBind Microcentrifuge Tubes) and store at -80 °C.
- ◆ Resuspend one aliquot in 50 µL of Solvent A (95% Optima H₂O, 5 % Optima Acetonitrile, 0.2% MS-grade formic acid), for a final concentration of 20 ng/µL.
- ◆ Mix thoroughly by pipetting, then transfer to a clean autosampler vial. The standard is now ready for use.
- ◆ A standard prepared in this way should be stable for up to three days at 4 °C (before significant protein oxidation becomes evident).

❖ LC Parameters

- ◆ **Solvent A:** 0.1% MS-grade formic acid in Optima H₂O
- ◆ **Solvent B:** 0.1% MS-grade formic acid in Optima Acetonitrile
- ◆ **Injection Volume:** 5 µL (100 ng loaded)

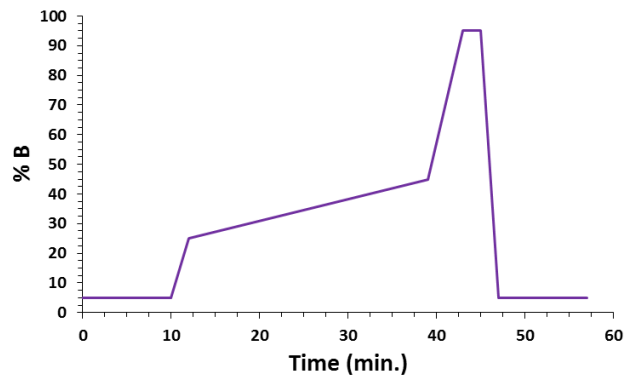
◆ **Column Parameters**

- Packing Material: PLRP-S resin, 1000 Å pore size, 5 µm particle size (obtained from Agilent Technologies)
- Trap column: 2 cm bed length, 150 µm I.D. (Self-packed)
- Analytical column: 15 cm bed length, 75 µm I.D. (Self-packed)
- Nanospray Emitter: 15 µm PicoTip emitter, packed with 2 mm PLRP-S resin (P/N FS360-50-15-N-20-C12, New Objective)

◆ **Gradient Parameters**

- Trapping Configuration: 3 µL/min flow rate (10 min. trap cycle, 45 °C)
- Analytical Configuration: 0.3 µL/min flow rate (48 min. analytical gradient, 45 °C)
- LC Gradient:

Time (min.)	% B	Curve
0.0	5.0	
10.0	5.0	0% in 10 min.
12.0	25.0	20% in 2 min.
39.0	45.0	20% in 27 min.
43.0	95.0	50% in 4 min.
45.0	95.0	0% in 2 min.
47.0	5.0	90% in 2 min.
57.0	5.0	0% in 10 min.



❖ **MS Parameters**

◆ **Instrument Tuning and Method Parameters:**

(All in positive and profile mode, with 15.0 V source CID and “Protein Mode” on.)

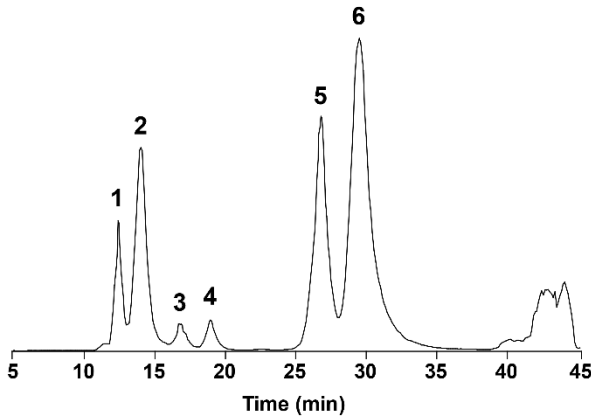
Scan Event 1: FTMS1	Scan Range (<i>m/z</i>)	500.00 – 2000.00
(15k RP)	Microscans	10
Full Scan	Max Inject Time (ms)	50.00
Normal mass range	MS1 AGC Target	1.00e +06
Scan Event 2: FTMS2	Activation type	HCD
Scan range	Define <i>m/z</i> range	400-2000
(60k RP)	Default charge state	10
Quadrupole isolation ON	Isolation Width (<i>m/z</i>)	4.0
Top 2, dd	Normalized Collision Energy	25.0
	Microscans	4
	Max Inject Time (ms)	800.00
	MS2 AGC Target	1.00e+06

◆ **Dynamic Exclusion Settings (MS2):**

Underfill Ratio	0.5 %
Exclude Isolation (Charge states 1-5)	On
Exclusion Duration (s)	60
Intensity Threshold	6.30e+03

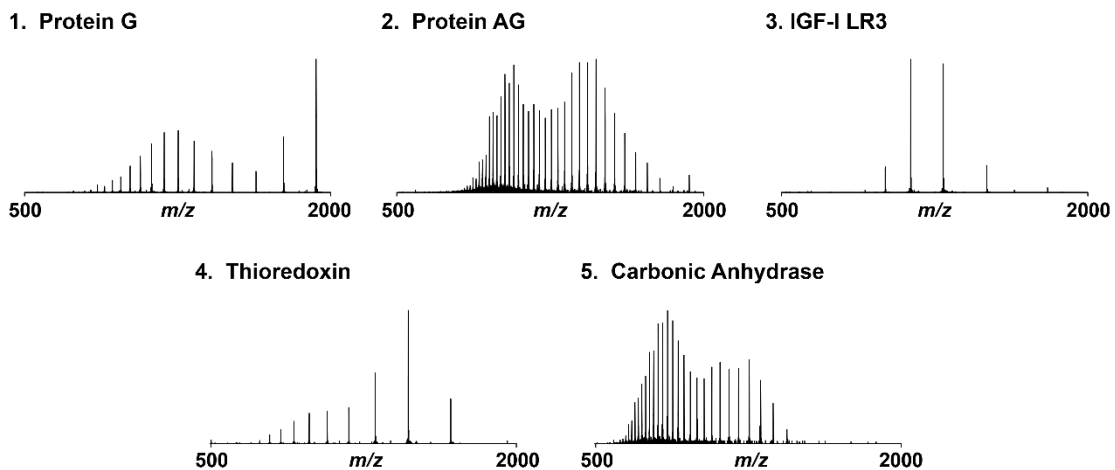
❖ **Data Interpretation and Analysis**

◆ **Example Chromatogram:**



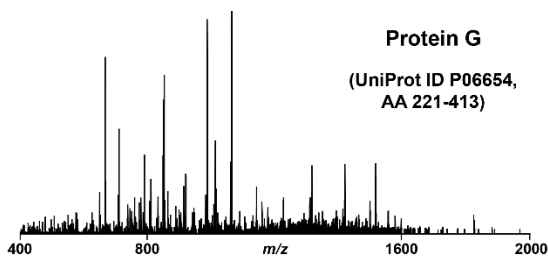
Example Chromatograms: Typical FT MS1 base peak chromatogram of the Pierce Intact Protein Standard on a PLRP-S column, showing six separate eluted protein peaks (**1.** Protein G, **2.** Protein AG, **3.** IGF-I LR3, **4.** Thioredoxin, **5.** Carbonic Anhydrase II, **6.** Exo Klenow). The elution order and relative height ratio of all protein peaks should remain consistent. The examples shown were obtained on a QE-HF, using the LC and MS parameters described above.

◆ **Example FT MS1 spectra**



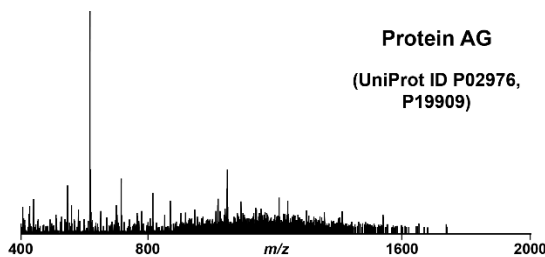
Example FT MS1 spectra: Averaged FTMS1 spectra for each of the six peaks in the above chromatogram, showing the characteristic isotopic peak distributions for each protein.

◆ Example FT MS2 spectra:



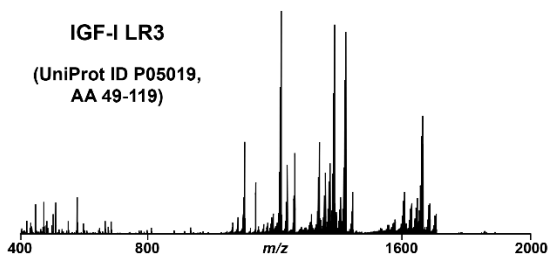
N M D P Y P L P K T D T Y K L I L N G K T L K G E T 25
 26 T T E A V D A A T A E K V F K Q Y A N D N G V D I G 50
 51 E W T Y D D A T K T F T V T E K P E V I D A S E L 75
 76 T P A V T T Y K L V I N G K T L K G E T T T K A V 100
 101 D A E T A E K A F K Q Y A N D N G V D G V W T Y D 125
 126 D A T K T F T V T E M V T E V P G D A P T E P E K 150
 151 P E A S I P L V P L T P A T P I A K D P A K K D D 175
 176 L T K K E L D A K K P E A K K D D A K K A E T A G C

P-score: 4 E-38, PCS: 395, 37 matching fragments



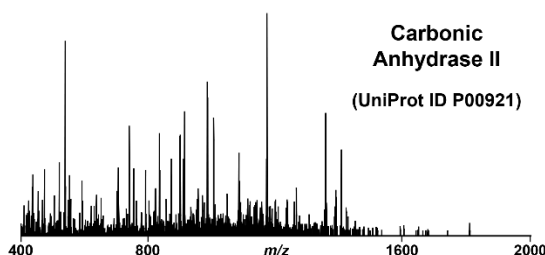
N A Q H D E A Q Q N A F Y Q V I L N M P N L N A D Q R 25
 26 N G F I Q S L K D D P S Q S A N V L G E A Q K L N 50
 51 D S Q A P K A D A Q Q N N F N K D Q Q S A F Y E I 75
 76 L N M P N L N E A Q R N G F I Q S L K D D P S Q S 100
 101 T N V L G E A K K L N E S Q A P K A D N N F N K E 125
 126 Q Q N A F Y E I L N M P N L N E E Q R N G F I Q S 150
 151 L K D D P S Q S A N L L S E A K K L N E S Q A P K 175
 176 A D N K F N K E Q Q N A F Y E I L H L P N L N E E 200
 201 Q R N G F I Q S L K D D P S Q S A N L L A E A K K 225
 226 L N D A Q A P K A D N K F N K E Q Q N A F Y E I L 250
 251 H L P N L T E E Q R N G F I Q S L K D D P S V S K 275
 276 E I L A E A K K L N D A Q A P K E E D N N K P I E 300
 301 G R N S R G S V D A S E L T P A V T T Y K L V I N 325
 326 G K T L K G E T T E A V D A A T A E K V F K Q Y 350
 351 A N D N G V D G E W T Y D D A T K T F T V T E K P 375
 376 E V I D A S E L T P A V T T Y K L V I N G K T L K 400
 401 G E T T T K A V D A E T A E K A F K Q Y A N D N G 425
 426 V D G V W T Y D D A T K T F T V T E M V T E V P L 450
 451 E S T A C

P-score: 8 E-16, PCS: 122, 14 matching fragments



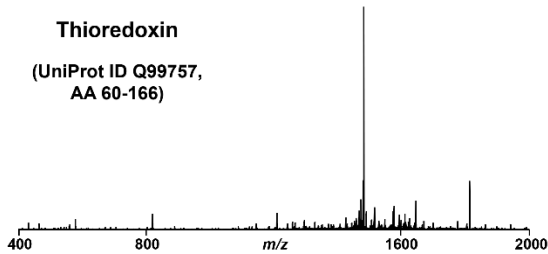
N M F P A M P L S L F V N G P R T L G A E L V D 25
 26 A L Q F V G D R G F Y F N K P T G Y G S S S R R 50
 51 A P Q T G I V D E C C F R S C D L R R L E M Y C A 75
 76 P L K P A K S A C

P-score: 2 E-14, PCS: 106, 20 matching fragments



N S H H W G Y G K H N G P E H W H K D F P I A N G E 25
 26 R Q S P V D I D I T K A V V I Q D P A L K P L A L V Y 50
 51 G E A T S R R R M V N N G H S F N V E Y D D S Q D K 75
 76 A V L K D G P L T G T Y R L V Q F H F H W G S S D 100
 101 D Q G S E H T V D R K K Y A A E L H L V H W N T K 125
 126 Y G D F G T A A Q Q P D G L A V G V F L K V G D 150
 151 A N P A L Q K V L D A L D S I K T K G K S T D F P 175
 176 N F D P G S L L P N V L N Y W T Y P G S L T P P 200
 201 L L E S V T W I V L K E L P I S V S S Q Q M L K F R 225
 226 T L N F N A E I G E P E L L L M L A N W R P A Q P L K 250
 251 N R Q V R G F P K C

P-score: 2 E-57, PCS: 641, 43 matching fragments



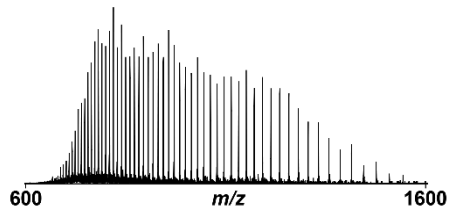
N T T F N I I Q D I G P D F Q D R V V N S E T P V I V I D 25
 26 F H A Q W G P C K I L G P R L E K M V A K Q H G 50
 51 K V V M A K V D I D D H T D L A I E Y E V S A V P 75
 76 T V L A M K N G D V V D K F V G I K I D E D I Q L L E A 100
 101 F L L K K L I G C

P-score: 3 E-64, PCS: 729, 70 matching fragments

Example FT MS2 spectra: Single-scan fragmentation spectra for Protein G, Protein AG, IGF-I LR3, Thioredoxin, and Carbonic Anhydrase II from the dataset shown above. The fragment ion masses from each of the above spectra were deconvoluted using the Xtract algorithm (Thermo) and searched against the respective protein sequences using ProSight Lite.

◆ **Advanced Parameters for Analysis of Exo Klenow**

6. Exo Klenow



For analysis of higher-MW proteins such as Exo Klenow, certain advanced parameters are required in order to obtain a high-quality intact mass spectrum. These parameters are available through the Developer’s Kit and described in detail in Fornelli et al., “Advancing Top-down Analysis of the Human Proteome Using a Benchtop Quadrupole-Orbitrap Mass Spectrometer”, *J. Proteome Res.*, 2017. An abbreviated summary is provided below.

“Medium-High” FTMS1	Scan Range (<i>m/z</i>)	500.00 – 2000.00
(7.5k RP at 200 <i>m/z</i>)	Microscans	25
Full Scan	Max Inject Time (ms)	50.00
Normal mass range	MS1 AGC Target	1.00e +06

◆ **Data Analysis Methods:**

- **ProSight Lite:** The software is available for free download at <http://prosightlite.northwestern.edu/>. A detailed protocol for the analysis of simple protein mixtures with Xtract and ProSight Lite can be found at https://link.springer.com/content/pdf/10.1007%2F978-1-4939-6783-4_18.pdf
- **ProSight PC 4.0:** A “Standards” search database for high-throughput data analysis of the Pierce Intact Protein Standard with ProSight PC 4.0 is available for download here: <http://proteinaceous.net/database-warehouse/>
- **NRTDP TDPortal:** A custom workflow for high-throughput analysis of the Pierce Intact Protein Standard is available on the TDPortal Quest-based, high-performance computing environment available through NRTDP and Northwestern University. User accounts can be requested at <http://nrtdp.northwestern.edu/tdportal-request/>. A detailed protocol for data analysis on TDPortal by external users (**NRTDP SOP_004**) can be found at <http://nrtdp.northwestern.edu/wp-content/uploads/2017/01/ExternalUserJan10.pdf>

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