

## SOP\_001\_NU\_1\_2\_Top\_Down\_Standard\_v2C\_LF\_CJD

Luca Fornelli and Caroline J. DeHart

## ❖ 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
MabPac RP Column	302598	Thermo 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

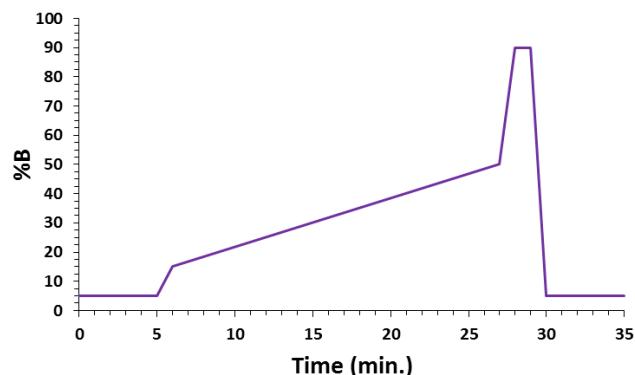
- ◆ Dilute one 76 µg vial into 100 µL of Solvent A (95% Optima H<sub>2</sub>O, 5 % Optima Acetonitrile, 0.2% MS-grade formic acid), for a final concentration of 0.76 µg/µL.
- ◆ Mix thoroughly by pipetting, then transfer to a clean autosampler vial. The standard is now ready for use.
- ◆ A standard prepared 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 H<sub>2</sub>O
- ◆ **Solvent B:** 0.1% MS-grade formic acid in Optima Acetonitrile
- ◆ **Column Parameters:**
  - 4 µm pore size, 1.0 mm I.D., 150 mm length
  - Injection volume: 1.5 µL (1.14 µg loaded)

- Gradient Parameters: 150  $\mu$ L/min flow rate, with temperature maintained at 60 °C

Time (min.)	% B	Curve
0.0	5.0	
5.0	5.0	0% in 5 min.
6.0	15.0	10% in 1 min.
27.0	50.0	35% in 21 min.
28.0	90.0	40% in 1 min.
29.0	90.0	0% in 1 min.
30.0	5.0	85% in 1 min.
35.0	5.0	0% in 5 min.



❖ **MS Parameters**

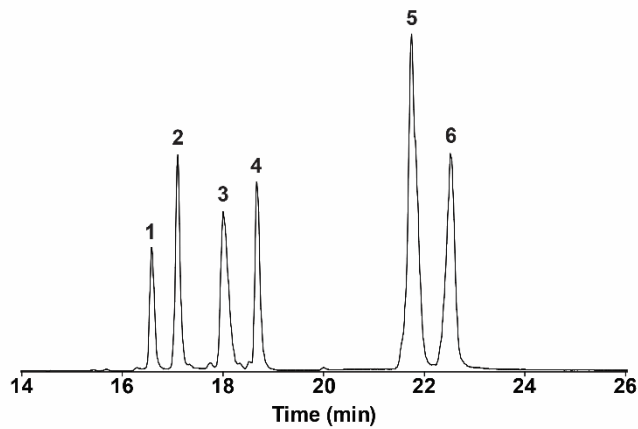
- ◆ **Instrument Tuning and Method Parameters:**

(All in positive and profile mode, with 15.0 V source CID. “Protein mode” on, set at 2 mtorr.)

<b>FT MS1 Parameters</b>	Scan Range ( <i>m/z</i> )	500.00 – 2000.00
(15k RP)	Microscans	5
Full Scan	Max Inject Time (ms)	50.00
Normal mass range	MS1 AGC Target	5.00e +05
<b>FT MS2 Parameters</b>	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> )	3.0
Top 2, dd	Normalized Collision Energy	22.0
Isolation offset OFF	Microscans	4
Supplemental activation OFF	Max Inject Time (ms)	800.00
Charge Filter: $6 \leq z \leq 24$	MS2 AGC Target	2.00e+05

❖ Data Interpretation and Analysis

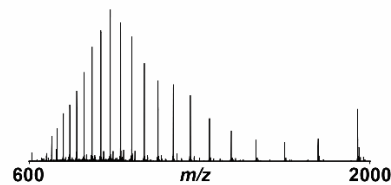
◆ Example Chromatogram:



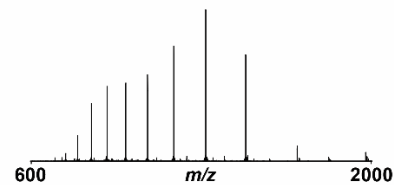
**Example Chromatograms:** Typical FT MS1 base peak chromatogram of the Pierce Intact Protein Standard on the MabPac RP column, showing six separate eluted protein peaks (**1.** Protein G, **2.** Protein AG, **3.** IGF-I LR3, **4.** Thioredoxin, **5.** Carbonic Anhydrase II, **6.** Exo Klenow). The elution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

◆ Example FT MS1 spectra:

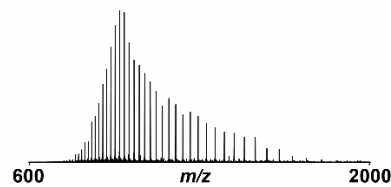
1. Protein G (21 kDa)



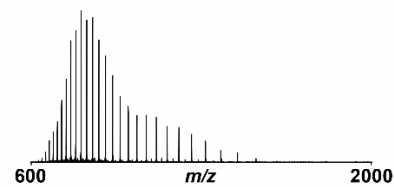
4. Thioredoxin (12 kDa)



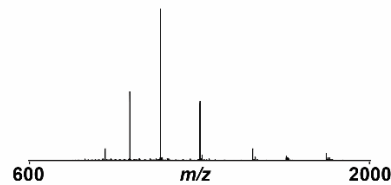
2. Protein AG (50 kDa)



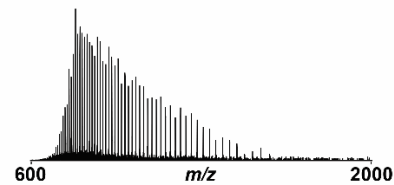
5. Carbonic Anhydrase II (29 kDa)



3. IGF-I LR3 (9 kDa)

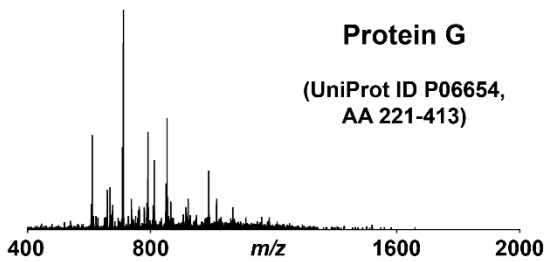


6. Exo Klenow (68 kDa)



**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:

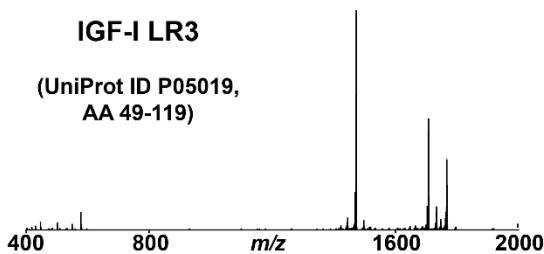


N M D P Y P L P K T D T Y K L I L N G K T L K G E T 25  
 26 T T E [A] V D [A] A T A E K V F K Q Y A N D N G [V] D [G] 50  
 51 E W T Y D D A T K T F T V T E K P E V I D A S E L 75  
 76 T P A V T T Y K L V I N G K T L K G E T T T K A V 100  
 101 D A E T A E K A F K Q Y A N D N G V D G V W T Y D 125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

P-score: 1 E-39, PCS: 415, 35 matching fragments

**IGF-I LR3**

(UniProt ID P05019, AA 49-119)

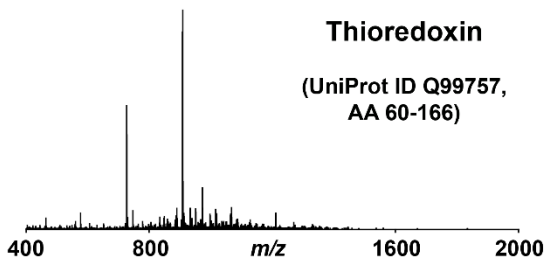


N M [F] [P] [A] M [P] [L] [S] [S] L F V N [G] P R T L [G] A E L V D 25  
 26 A L Q F V [G] D R G F F Y F N K P T G Y G S S S R R 50  
 51 A P Q T G I V D E [G] [G] F R S [G] D L R R L E M Y [A] 75  
 76 P L K P [A] K S A C

P-score: 1 E-18, PCS: 156, 14 matching fragments

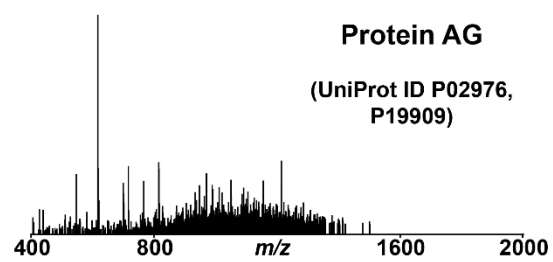
**Thioredoxin**

(UniProt ID Q99757, AA 60-166)



N T T F N [I] [Q] [D] [C] [P] D [F] Q D R [V] V N S [E] T P V V D 25  
 26 F H A Q W [G] G P [G] 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] [D] E D [Q] [L] E [A] 100  
 101 [F] [L] [K] [K] L I G C

P-score: 4 E-45, PCS: 484, 47 matching fragments

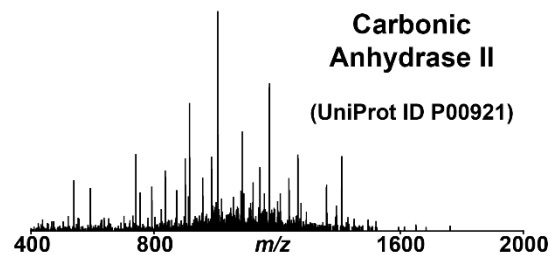


N A Q H D E A [Q] [N] [A] [F] [Y] [Q] [V] [L] N M P N L N A D Q R 25  
 26 N G F I Q S L K D D P S Q S A N V L G E A Q K L N 50  
 51 D S Q A P K A D A Q Q N N F N K D Q S A F Y E I 75  
 76 L N M P N L N E A Q R N G F I Q S L K D D P S Q S 100  
 101 T N V L G E A K K L N E S Q A P K A D N N F N K E 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 T E A V D 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: 7 E-14, PCS: 100, 13 matching fragments

**Carbonic Anhydrase II**

(UniProt ID P00921)



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] [T] [K] A [V] [Q] [D] [P] [A] [L] [K] P L A L V Y 50  
 51 G E A T S 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 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 P N V L N Y W T [V] [P] G S L T [T] [P] [P] 200  
 201 L L E S V T W I V L K [E] [P] I S V S S [Q] [M] [L] [R] F R 225  
 226 T L N F N A E [G] [E] [P] E [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: 7 E-41, PCS: 430, 44 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.

## ◆ 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](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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