SOP_001_NU_1_2_Top_Down_Standard_November_2015_v1_CJD_LF

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❖ Reagent and Materials List

Item	Part Number	Vendor
Ubiquitin	U6253	Sigma Aldrich
Trypsinogen	T1143	Sigma Aldrich
Myoglobin	M5696	Sigma Aldrich
Carbonic Anhydrase	C2624	Sigma Aldrich
Optima Grade Water	W6	Fisher Scientific
Optima Grade Acetonitrile	A955	Fisher Scientific
MS-Grade Formic Acid	PI-28905	Fisher Scientific
1.5 mL Protein LoBind Microcentrifuge Tubes	13-698-794	Fisher Scientific

❖ Important Notes

- Use 1.5 mL Eppendorf LoBind microcentrifuge tubes for protein stock preparation, top-down (TD) standard preparation, and long term aliquot storage. In our experience, these tubes have shown the lowest degree of plasticizer leaching and/or protein binding during use and storage.
- Approximate final protein amounts (loaded on-column): 0.1 pmol ubiquitin, 0.5 pmol trypsinogen, 1 pmol myoglobin, and 0.6 pmol carbonic anhydrase. Superoxide dismutase (SOD1) is present as a contaminant in carbonic anhydrase.
- A TD standard prepared in this way should be stable for up to three days at 4 °C (before significant protein oxidation becomes evident).

❖ Recipe

- Prepare 2 mg/mL stocks of each protein standard in Optima H_2O. (Aliquots can be stored at -80 °C.)
- Prepare the following (volumes shown from respective stock solutions):

Protein	Volume (µL)	Stock Concentration (pmol/µL)	Amount Loaded on Column (pmol, 1X)
Carbonic Anhydrase	40	25.7	0.64
Myoglobin	40	43.9	1.09
Trypsinogen	25	19.6	0.49
Ubiquitin	2.5	5.5	0.14
Total	**107.5**		

- Divide final mixture into 2.5 uL aliquots and store at -80 °C.

❖ Preparation

- Dilute one aliquot of TD STD in 100x vol. of Buffer A (95% Optima H_2O, 5 % Optima Acetonitrile, 0.2% MS-grade formic acid), where 1x vol. is the intended injection volume (e.g. **600 µL Buffer A** for an intended injection volume of **6 µL**). This will ensure that the correct amount of each TD standard protein is present in each injection.
- Mix thoroughly by pipetting, then transfer to a clean autosampler vial. The standard is now ready for use.

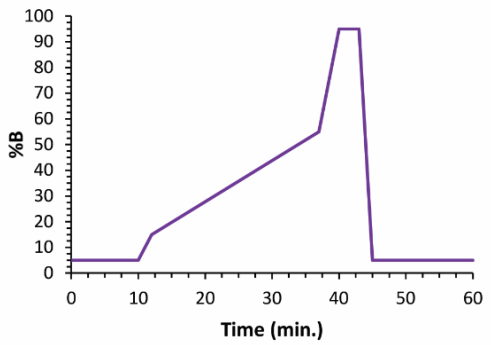
❖ Column Parameters

- **Option 1**: Self-packed
  - 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.
  - Analytical column: 15 cm bed length, 75 µm I.D.
  - Nanospray Emitter: 15 µm PicoTip emitter, packed with 2 mm PLRP-S resin (P/N FS360-50-15-N-20-C12, New Objective)
- **Option 2**: Purchased from New Objective
  - Trap column: 1 cm bed length, 150 µm I.D. New Objective Integrafrit (P/N IF150-10H502)
  - Analytical column: 15 cm bed length, 75 µm I.D. New Objective PicoFrit (P/N PF7515-150H502)

❖ LC Parameters

- Solvent A: 95% Optima H_2O, 5% Optima Acetonitrile, 0.2% MS-grade formic acid
- Solvent B: 5% Optima H_2O, 95% Optima Acetonitrile, 0.2% MS-grade formic acid
- Trapping configuration: 3 µL/min flow rate (10 min. trap cycle)
- Analytical configuration: 0.3 µL/min flow rate (50 min. analytical gradient)
- Gradient Parameters:

Retention Time (min.)	% B	Curve
0.0	5.0	
10.0	5.0	0% in 10 min.
12.0	15.0	10% in 2 min.
37.00	55.0	40% in 25 min.
40.00	95.0	40% in 3 min.
43.00	95.0	0% in 3 min.
45.00	5.0	90% in 2 min.
60.00	5.0	0% in 15 min.



❖ **MS Parameters**

- **LTQ-Velos FT-ICR (12T):**

  - Instrument Tuning and Method Parameters:
    (All in positive and profile mode, with 15.0 V source CID)

Scan Event 1: FTMS1	Scan Range (*m/z*)	500.00 – 2000.00
(100k RP)	Microscans	4
Full scan	Max Inject Time (ms)	2000.00
Normal scan rate	MS1 AGC Target	1.00e +06
Scan Event 2: FTMS2	Minimum Signal Threshold (counts)	500
(50k RP)	Activation Type	CID
Top 1, dd	Default Charge State	10
Full scan	Isolation Width (*m/z*)	15.0
Normal scan rate	Normalized Collision Energy	41.0
	Activation Q	0.400
	Activation time (ms)	100.00
	Microscans	4
	Max Inject Time (ms)	2000.00
	MS2 AGC Target	1.00e +06
Scan Event 3: ITMS1	Scan Range (*m/z*)	500.00- 2000.00
Full scan	Microscans	25
Normal scan rate	Max Inject Time (ms)	50.0
	MS1 AGC Target	3.00e + 04

- **LTQ-Velos Orbitrap Elite:**

  - Instrument Tuning and Method Parameters:
    (All in positive and profile mode, with 15.0 V source CID. Protein mode on.)

Scan Event 1: FTMS1	Scan Range (*m/z*)	500.00 – 2000.00
(120k RP)	Microscans	4
Full Scan	Max Inject Time (ms)	100.00
Normal scan rate	MS1 AGC Target	1.00e +06
Scan Event 2: ITMS1	Scan Range (*m/z*)	500.00- 2000.00
Full Scan	Microscans	25
Normal scan rate	Max Inject Time (ms)	10.00
	MS1 AGC Target	3.00e +04
Scan Event 3: FTMS2	Minimum Signal Threshold (counts)	500
(60k RP)	Activation Type	HCD
Top 1, dd	Default Charge State	10
Full Scan	Isolation Width (*m/z*)	15.0
Normal scan rate	Normalized Collision Energy	23.0
	Activation Q	0.250

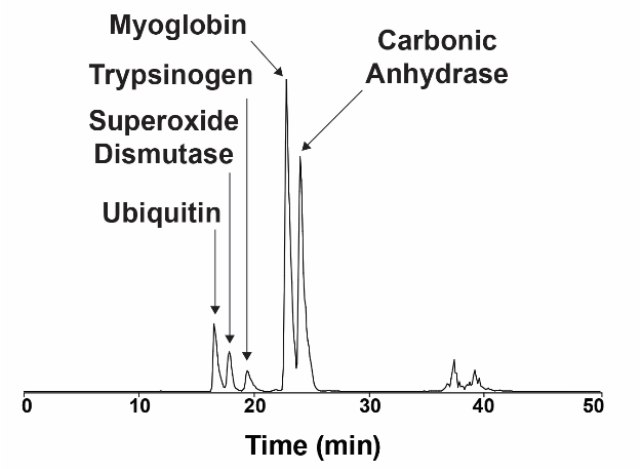
Scan Event 3: FTMS2	Activation time (ms)	0.1
(continued)	Microscans	4
	Max Inject Time (ms)	1000.00
	MS2 AGC Target	1.00e +06

- **Dynamic Exclusion Settings (MS2) for both instruments:**

Repeat Count	1
Repeat Duration (s)	30.0
Exclusion List Size	50
Exclusion Duration (s)	90
Exclusion Mass Width (High/Low)	1.50

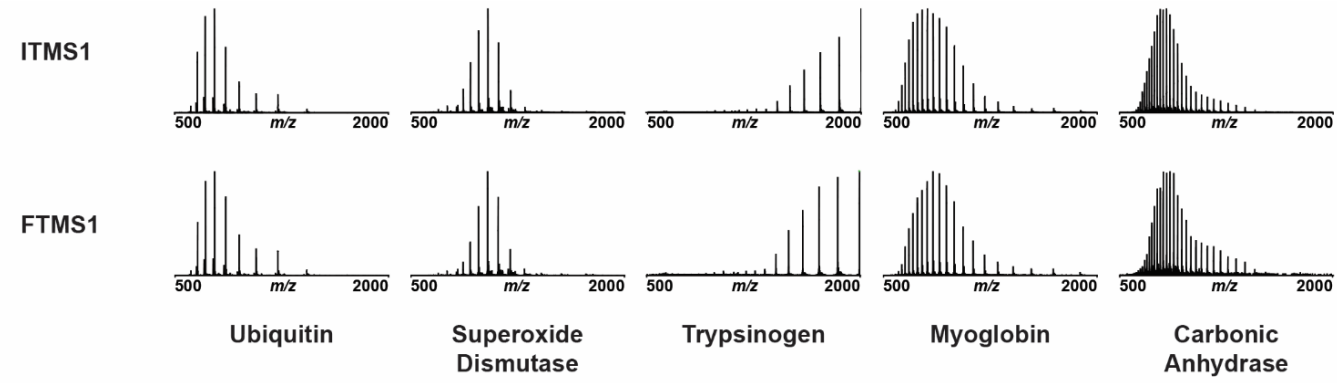
❖ Data Interpretation and Analysis

- **Example Chromatogram:**



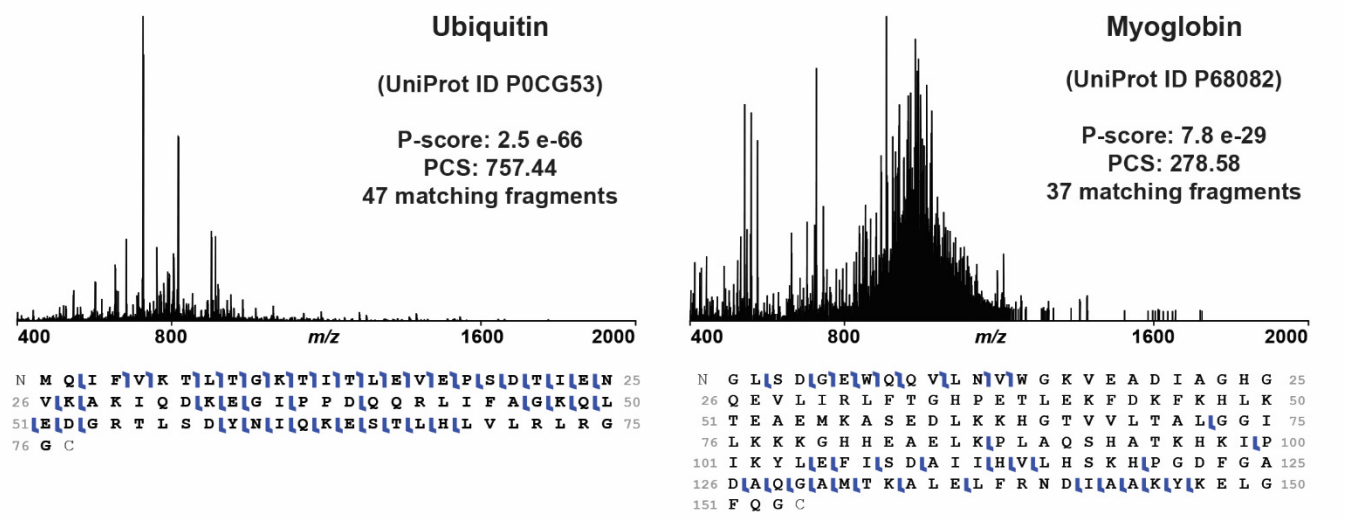
Example Chromatogram: Typical base peak chromatogram of the NRTDP TD standard, showing five separate eluted protein peaks (the fifth being due to superoxide dismutase, a characteristic contaminant of carbonic anhydrase). The elution order and relative height ratio of each protein peak should remain consistent. The example shown was obtained on the LTQ-Velos Orbitrap Elite, using the LC and MS parameters described above.

- **Example IT and FT MS1 spectra:**



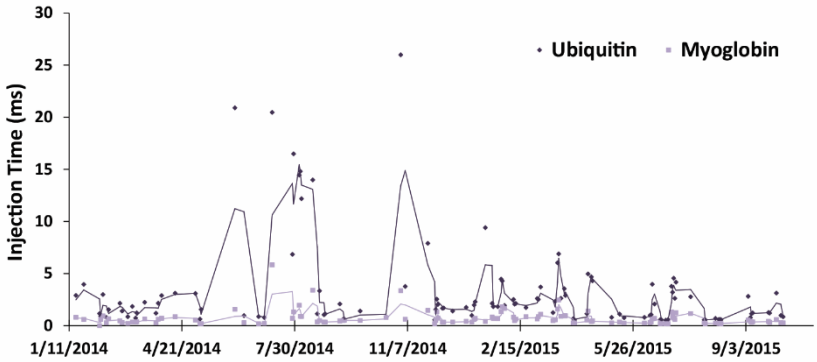
Example IT and FT MS1 spectra: Averaged ITMS1 and FTMS1 spectra for each of the five peaks in the above chromatogram (LTQ-Velos Orbitrap Elite), showing the characteristic isotopic peak distributions for each protein. Note the fidelity of the FTMS1 spectra to those acquired in the ion trap; this serves as an indicator of optimal ion transmission and FT performance.

- **Example FT MS2 spectra:**



Example FT MS2 spectra: Example single-scan fragmentation spectra for ubiquitin (**left**) and myoglobin (**right**) from the above chromatogram (LTQ-Velos Orbitrap Elite). 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 (available for free download at http://prosightlite.northwestern.edu/). Typical P-scores for ubiquitin should be below E -50, while P-scores for myoglobin should be below E -25.

- **Example use of TD standard data for longitudinal tracking of instrument performance:**



Example longitudinal data: Consistent observation of certain metrics, in this case the MS1 injection time (ms) of ubiquitin and myoglobin in TD standards run on the LTQ-Velos Orbitrap Elite, can indicate the onset of ion optics charging or other instrument issues that might otherwise be more difficult to detect.

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