Apr 08, 2021

NRTDP Top Down Standard SOP: Orbitrap Eclipse and Exploris

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1 Works for me dx.doi.org/10.17504/protocols.io.bt27nqhn

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ABSTRACT

This standard protocol details how to make a standard mix of proteins for tracking LC-MS performance and illustrates how to configure an instrument for high-quality data collection.

This protocol was developed with support from the National Institute of General Medical Sciences P41GM108569 for the National Resource for Translational and Developmental Proteomics (NRTDP) based at Northwestern University. The authors thank Melinda Wojtkiewicz from the University of Nebraska Medical Center for helping us troubleshoot this method for use with the Exploris 480 in the Gundry Lab.

EXTERNAL LINK

http://nrtdp.northwestern.edu/protocols/

DOI

dx.doi.org/10.17504/protocols.io.bt27nqhn

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PROTOCOL CITATION

Rafael Melani, Paul Thomas, Bryon Drown 2021. NRTDP Top Down Standard SOP: Orbitrap Eclipse and Exploris. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bt27nqhn

KEYWORDS

top-down mass spectrometry, protein mass spectrometry, proteomics, top-down proteomics

CREATED

Apr 07, 2021

LAST MODIFIED

Apr 08, 2021

OWNERSHIP HISTORY

Apr 07, 2021 Kelleher Research Group Northwestern University

Apr 08, 2021 Nrtdp Help

PROTOCOL INTEGER ID

48959

MATERIALS TEXT

Ubiquitin (Sigma Aldrich, U6253)
Trypsinogen (Sigma Aldrich, T1143)
Myoglobin (Sigma Aldrich, M5696)
Carbonic anhydrase (Sigma Aldrich, C2624)
Optima Grade Water (Fisher Scientific, W6-4)
Optima Grade Acetonitrile (Fisher Scientific, A955-4)
MS-Grade Formic Acid (Fisher Scientific, PI-28905)
1.5 mL Protein LoBind Microcentrifuge Tubes (Fisher Scientific, 13-698-794)

PLRP-S resin, 1000 Å pore size, 5 µm particle size (obtained from Agilent Technologies)
15 µm PicoTip emitter, packed with 2 mm PLRP-S resin (P/N FS360-50-15-N-20-C12, New Objective)

Solvent A: 95% Optima H2O, 5% Optima Acetonitrile, 0.2% MS-grade formic acid
Solvent B: 5% Optima H2O, 95% Optima Acetonitrile, 0.2% MS-grade formic acid

BEFORE STARTING

- 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).

Prepare Standard Mix

1 Prepare **2 mg/ml** stocks of each protein standard in Optima water. (Aliquots can be stored at **-80 °C**)

2 Prepare the following mixture (volumes shown from respective stock solutions):

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

Preparation of protein standard mixture

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3 Divide final mixture into **2.5 µl** aliquots and store at **-80 °C**

4 When ready for acquisition, dilute one aliquot of TD STD in 100x vol. of Buffer A (95% Optima water, 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.

LC Parameters

5 Prepare and condition a self-pack PLRP-S column
- 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: 20 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)

6 Inject **6 µl** onto trap column and execute the following gradient.
- Solvent A: 95% Optima H2O, 5% Optima Acetonitrile, 0.2% MS-grade formic acid
- Solvent B: 5% Optima H2O, 95% Optima Acetonitrile, 0.2% MS-grade formic acid

Trapping configuration: 3 µL/min flow rate (10 min. trap cycle, 55 °C)
Analytical configuration: 0.3 µL/min flow rate (48 min. analytical gradient, 55 °C)

A	B	C
Time (min.)	**% B**	**Curve**
0	5	
10	5	0% in 10 min
14	15	10% in 4 min
40	45	30% in 26 min
42	95	50% in 2 min
46	95	0% in 4 min
50	5	90% in 4 min
60	5	0% in 10 min

LC gradient

MS Acquisition

7 The mass spectrometer was operated in the following configuration:
Positive and profile mode, RF Lens 30%, with 15.0 V source CID. “Intact Protein” on if available, “Low Pressure” selected if available, Default Charge State 15, “Advanced Peak Determination” on

8 **Method Parameters:**

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A	B	C
Experiment 1		
Master Scan: FTMS1	Scan Range (m/z)	600-2000
(120k RP)	Microscans	1 (2 if no low-pressure mode)
Full Scan	Max Inject Time (ms)	100
Normal mass range	MS1 AGC Target	1.2e6 (300%)
Scan Event 1: ddMSNScan	Activation type	HCD
Scan range	Define m/z range	350-2000
(60k RP)		
Quadrupole ioslation ON	Isolation width (m/z)	3
Top N 3 sec, dd	Normalized Collision Energy	28
Isolation offset, OFF	Microscans	1 (2 if no low-pressure mode)
Supplemental activation OFF	Max Inject Time (ms)	600
Charge Filter: 5 < z < 30	MS2 AGC Target	5e5 (1000%)
Experiment 2 (Eclipse Only)		
Master Scan: ITMS1	Scan Range (m/z)	600-2000
Full Scan	Microscans	20
Rapid scan rate	Max Inject Time (ms)	10
Normal mass range	MS1 AGC Target	3e4

Dynamic Exclusion Settings (MS2):

A	B
Repeat Count	1
Exclusion Duration (s)	60
Exclusion Mass Width (High/Low, m/z)	0.5
Exclude Isotopes	True

Data Interpretation Analysis

9 Example Data

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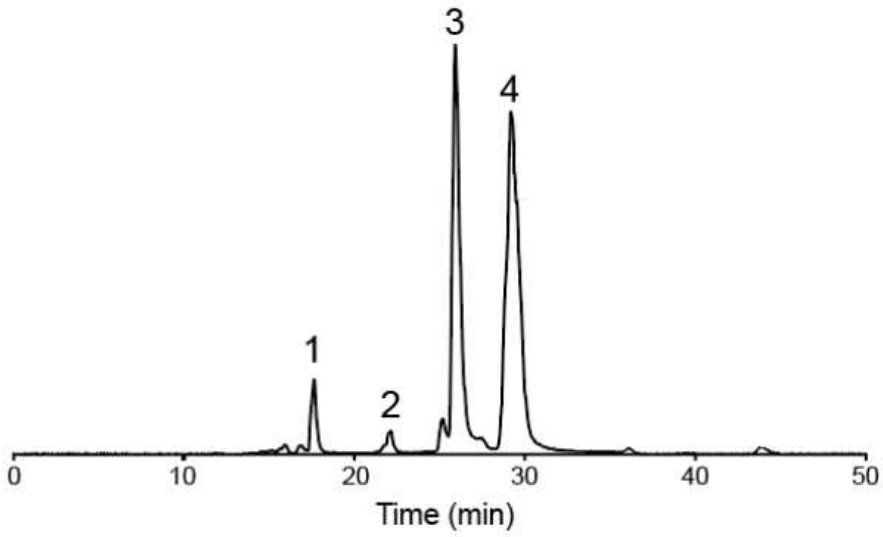
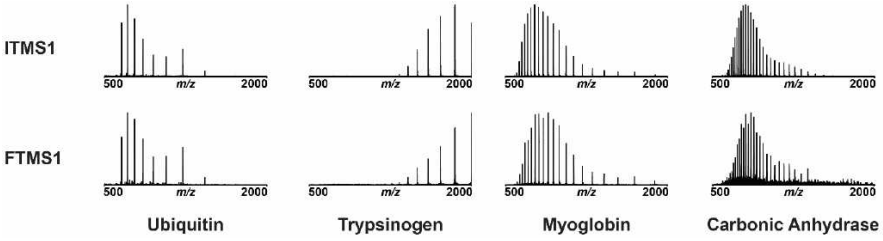


Example chromatograms: Typical base peak chromatogram of the NRTDP TD standard on column described above, showing four separate eluted protein peaks (1. Ubiquitin, 2. Trypsinogen, 3. Myoglobin, 4. Carbonic Anhydrase). Superoxide dismutase, a characteristic contaminant of carbonic anhydrase, is not present in these batches of TD standard. The elution order and relative height ratio of all other protein peaks should remain consistent.



Example IT (Eclipse) and FT MS1 spectra: Averaged ITMS1 and FTMS1 spectra for each of the four peaks in the above chromatogram, 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 for the Eclipse.

10 Data analysis methods:

- **ProSight Lite**: The software is available for free download at http://prosightlite.northwestern.edu/. A detailed protocol for the analysis of the NRTDP Top-Down Standard 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 NRTDP Top-Down 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 NRTDP Top Down 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/tdportalrequest/. 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

11 Longitudinal Data Tracking

The NRTDP recommends including the following metrics into longitudinal tracking of LC and MS performance:

- **Peak Intensity**: IT and FT MS1 peak intensity of ubiquitin, myoglobin, and carbonic anhydrase
- **Peak Area (log)**: Chromatographic peak area (and retention time) of ubiquitin, myoglobin, and carbonic anhydrase
- **FWHM**: Full width at half maximum of ubiquitin, myoglobin, and carbonic anhydrase peaks
- **Injection Time**: MS1 and MS2 injection times for ubiquitin, myoglobin, and carbonic anhydrase

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- **P-score (-log)**: ubiquitin, myoglobin, and carbonic anhydrase obtained by low- or high-throughput data analysis

Paying close attention to these parameters over time can help identify LC or MS issues before they become significant, thus reducing loss of important sample data.

The NRTDP further recommends running at least three injections of the Top Down Standard before and after running experimental samples, as well as at least one injection of Top Down Standard every twenty-four hours. Evaluation of these standards on the fly will not only help detect LC or MS issues, but also provide confirmation that optimal performance is maintained when consistency and reproducibility are crucial.

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