LC-MS Sample Preparation: Automated Protein Digestion and Clean-up on the Opentrons Flex®

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INTRODUCTION

Mass spectrometry-liquid chromatography (LC-MS) is a critical tool for characterizing the proteome. However, workflows, which involve digesting proteins into peptides, separating by LC, and identifying with MS, are time-consuming and can be technically challenging. After purification, quantification, and normalization, protein digestion and sample clean-up are required to prepare the samples for LC-MS analysis **(Figure 1)**. Automating these workflows can increase both throughput and reproducibility. Here, two protocols for the Opentrons Flex liquid handling robot were used to automate protein digestion and single-pot, solid-phase-enhanced (SP3) sample clean-up.

METHODS AND RESULTS

Two publicly available protocols were used to automate trypsin digestion and sample clean-up on the Opentrons Flex **(Figure 2)**. The protein digestion protocol first mixed 10 µg of monoclonal antibody (purified recombinant rabbit IgG dissolved in 100 µL of 100 mM ammonium bicarbonate) with 10 µL of dithiothreitol (DTT; 60 mM), followed by a 30-minute incubation at 55 °C in the on-deck thermocycler to denature protein samples. Next, 10 µL of iodoacetamide (IAA; 187.5 mM) was added to each sample, followed by another 30-minute incubation at room temperature to allow for alkylation. Finally, 10 µL of MS-grade trypsin (0.2 µg/µL) was transferred to each sample, which were then incubated for16 hours at 37 °C in the on-deck thermocycler to allow for in-solution trypsin digestion.

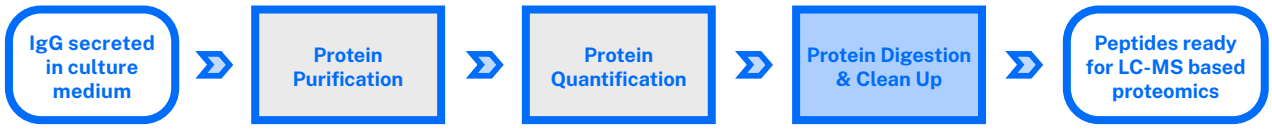
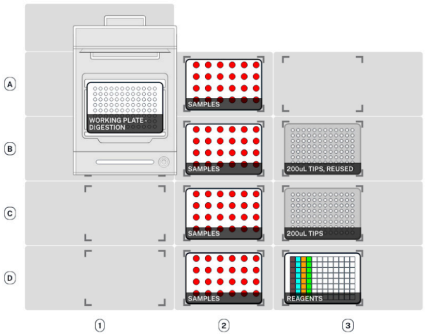
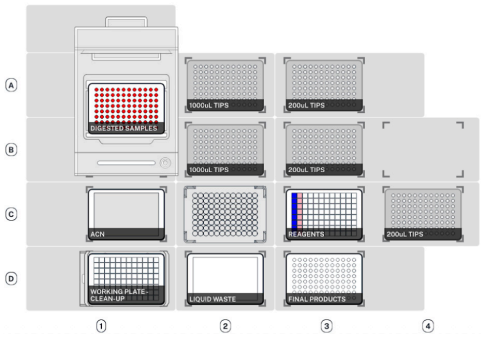


Figure 1. Sample preparation workflow automated on the Opentrons Flex. In this application note, we detail the third step in the process, protein digestion and clean-up. See other application notes in the series for more details on protein purification and protein quantification and normalization.



Trypsin Digestion for LC-MS

- Slot A1 and B1 - Thermocycler Module GEN 2 with Opentrons Tough 96 Well Plate 200 µL PCR Full Skirt
- Slot A2 to D2 - Opentrons 24 Tube Rack with NEST 1.5 mL Snapcap or
- Slot A2 - Opentrons Tough 96 Well Plate 200 µLPCR Full Skirt
- Slot B3 - Opentrons Flex 96 Tip Rack 200 µL
- Slot C3 - Opentrons Flex 96 Tip Rack 200 µL



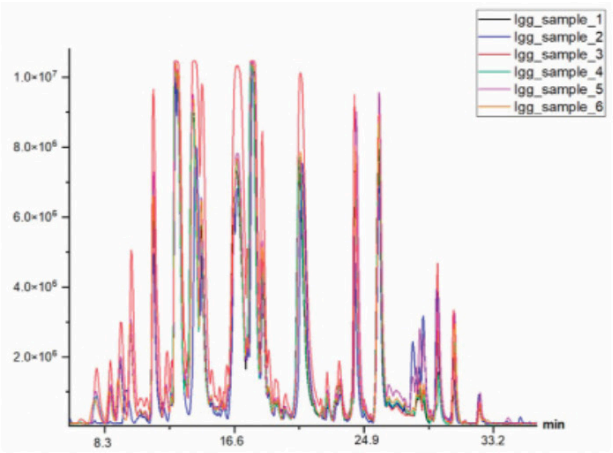
Digested Sample Clean-up for LC-MS

- Slot A1 and B1 - Thermocycler Module GEN 2 with Opentrons Tough 96 Well Plate 200 µL PCR Full Skirt (Digested Samples)
- Scot C1 - NEST 1 Well Reservoir 290 mL
- Slot D1 - Heater Shaker Module GEN1 with Opentrons 96 Deep Well Heater-Shaker Adapter and NEST 96 Deep Well Plate 2 mL
- Slot A2 - Opentrons Flex 96 Tip Rack 1000 µL
- Slot B2 - Opentrons Flex 96 Tip Rack 1000 µL
- Slot C2 - Magnetic Block GEN1
- Slot D2 - NEST 1 Well Reservoir 290 mL
- Slot A3 - Opentrons Flex 96 Tip Rack 200 µL
- Slot B3 - Opentrons Flex 96 Tip Rack 200 µL
- Slot C4 - Opentrons Flex 96 Tip Rack 200 µL
- Slot C3 - NEST 96 Deep Well Plate 2 mL

Figure 2. Deck setup for automated protein digestion and clean-up on the Opentrons Flex.

Immediately after protein digestion, the Opentrons Flex conducted an automated SP3 desalting protocol. Briefly, digested samples were transferred from the thermocycler to a new working plate on a heater-shaker module and mixed with 10 µL of bead slurry (Cytiva Sera-Mag carboxylate-modified magnetic particles; 50 µg/µL) and acetonitrile (ACN, 1292 µL) for peptide capture. Beads were collected by magnetic separation, followed by a wash step with ACN (1000 µL). Peptides were eluted in 2% DMSO (80 µL). Final products were vacuum dried off-deck.

LC-MS analysis reveals well-aligned base peak ion chromatography patterns across samples, with detected proteins relevant to subunits of rabbit IgG reliably detected **(Figure 3)**.



10 most abundant proteins in the samples

- 47 kDa protein, *Bos taurus*
- TREMBL:Q05B55, Similar to Ig kappa chain C region, *Bos taurus*
- TREMBL:Q1RMN8, Similar to Immunoglobulin lambda-like polypeptide 1, *Bos taurus*
- Trypsin, *Sus scrofa*
- Albumin, *Bos taurus*
- 12 kDa protein, *Bos taurus*
- Ig gamma chain C region, *Oryctolagus cuniculus*
- Ig kappa-b4 chain C region, *Oryctolagus cuniculus*
- Immunoglobulin G-binding protein G, *Streptococcus sp.*
- Immunoglobulin lambda-1 light chain, *Homo sapiens*

	Number of Proteins Detected					
	Sample #1	**Sample #2**	**Sample #3**	**Sample #4**	**Sample #5**	**Sample #6**
	33	37	41	34	34	39
***Oryctolagus cuniculus* IgG subunit**	**Score**					
Ig gamma chain C region	403	529	554	507	360	442
Ig gamma chain C region	65	413	497	356	181	262

Figure 3. Three batches of purified and normalized monoclonal antibody were digested and desalted in duplicate (Sample #1 and #4, Sample #2 and #5, Sample #3 and #6) and analyzed by Bruker's maXis-II ETD ESI-QqTOF/Dionex Ultimate-3000 LC system at the CUNY Advanced Science Research Center (New York, NY). Base peak intensity chromatograms (ion current vs. retention time) (upper left) and proteins detected (upper right) were presented.

CONCLUSION

The Opentrons Flex and publicly available protocols can be used to automate protein digestion and SP3 clean-up with desirable sample handling quality. This approach offers a convenient solution for routine tasks, leading to reduced labor requirements or allowing for the integration of sophisticated workflows in LC-MS-based proteomic analysis.