Analysis Report SP0014

Client		
Tel.		
E-mail		
Date	**Received:**	**Reported:**
Sample type		
Service type		
Operator		
Tel.		
Samples labeling (ID)		
Provided data		
Introduction		
Pipeline analysis	Non targeting proteomic analysis for the sample.	

Materials &Methods

1. Sample preparation

A. Protein Extraction and denaturation

1. 8M Urea with 10µl Protease inhibitor added on Control sample
2. Shake vigorously and centrifuge on 10,000 RPM or 30 minutes at 4 ^{0}C.

B. Protein quantification

1. To measuring concentration using bicinchoninic acid assay (BCA assay) as follows:

Sample	BSA (µl)	Sample (µl)	Sample Vehicle (µl)	MilliQ (µl)	BCA working solution (µl)
Blank	0	0	8	12	400
Standard (1.25 µg/ µl)	8	0	8	4	400
Sample	0	8	0	12	400

Sample	Conc. (ug/ul)	Total protein needed for digestion (30 ug)	
		Sample	**Urea Buffer (ul)**

			(ul)	**(Mass up to 30ul)**
		7.30	4.107	25.892
		5.40	5.547	24.452

C. Protein digestion	Directly digest the sample with the above volume.	
	For reduction	1. Add 2 µl of 200 mM DTT, vortex, and then spin down. 2. Incubate 30 min at RT.
	For Alkylation	1. Add 2µl of 1M IAA, and then incubate at room temperature for 45 min to 1 hour in dark. 2. Add 102 µl of 100mM Tris pH 8.5.
	For trypsinization	1. Add 6µl Trypsin containing 1ug procaine enzyme. 2. Incubate overnight at 37°C with shaking at 900 rpm. 3. Add 6ul of 100% Formic acid, to acidify the sample to pH 2-3. 4. Spin down for 30 min at max speed at room temperature.
D. Stage tip (Pierce™ C18 Spin Tips) prod#84850	For Activation	Add 15ul Methanol on the tip.
	For Initialization	Add 15ul from "solution B" (0.2% FA+ 80% ACN)
	For re-equilibration	Add 15ul from "solution A" (0.2% FA) twice.
	For sample trapping	Change the eppendorf tube and add the sample.
	For washing	Wash with 15µl "solution A" twice.
	For Elution	- In a collection tube add 3 times 20µl "solution B". - Speed-vac the sample then re-constitute in Solution A. - Inject the samples on Mass spectrometry.
	N.B: Centrifuge between each step at 3000 RPM in Stage tip	
E. Peptide quantification	To measure concentration using bicinchoninic acid assay (BCA assay) as follows:	

Sample	BSA (µl)	Sample (µl)	Sample Vehicle (µl)	MilliQ (µl)	BCA working solution (µl)
Blank	0	0	10	15	25
Sample	0	10	0	15	25
Standard (1 µg/ µl)	10	0	10	5	25

i. Incubate at 95 ^{0}C for 5 min.

ii. Add 1000 µl prepared BCA

iii. Incubate at 60 ^{0}C for 30 min.

iv. Then cool down at RT for 20 min.

v. Read at A_{562}.

2.Chromatography

LC system	NanoLC system consisting from Eksigent nanoLC 400 autosampler attached with Ekspert nanoLC425 pump.
Injection volume	10 µl (0.5 ug)
Injection mechanism	Trap and Elute
Needle wash	Two cycles using 10% isopropanol
Analysis time	55 minutes
Trapping parameters	Sample clean up using trapping cartridge CHROMXP C18CL 5um (10x0.5 mm) pumped at flow rate 10 ul/min for 3 min using mobile phase A
Column	3 um, ChromXP C18CL, 120A, 150 x 0.3mm
Flow rate	2 ul/min
Mobile phase	A) DI-Water containing 0.1 % FA B) Acetonitrile containing 0.1 %FA

Gradient profile	**Time (min) h,**	**% A**	**% B**
	0	97	3
	38	70	30
	43	60	40

	45	20	80
	48	20	80
	49	97	3
	57	97	3

Mass spectrometry	LC-QTOF system	Sciex TripleTOF™ 5600+
	Acquisition mode	Positive
	IDA parameters	High resolution TOF MS survey scan followed by product ion scan for the most abundant 40 ions. Cycle time is 1.5 sec.
	TOF mass range	400 – 1250 m/z
	MS2 range (product ion)	170 – 1500 m/z
	Ion selection threshold	150 cps
	Total run time	55 min
	MS calibration	Sciex tuning solution (P/N 4457953)
Data processing	- Analyst TF 1.7.1 is used for data acquisition (Sciex software). - Raw MS files from the TripleTOF™ 5600+ is converted into MGF files then analyzed by Peptide shaker (*v1.16.36).* - Database used is Aspergillus (swiss-prot and TrEMBL database containing 792561 proteins) - The search parameters used in searching in the following table	

3. **The search parameters**

The search parameters used in searching in the following table	Digestion	Trypsin	
	Identification Algorithms	X!Tandem	
	Max missed cleavages	2	
	Precursor m/z Tolerance	20.0 ppm	
	Fragment m/z Tolerance	10.0 ppm	
	Precursor Charge	2-5	
	Isotopes	0-1	
	Modification	**Fixed Modification**	**Variable Modification**

		Carbamidomethylation of C (Mass;57.02)	Acetylation of K (Mass;42.01)
			Acetylation of protein N-term (Mass;42.01)
			Deamidation of N ((Mass;0.98)
			Deamidation of Q (Mass;0.98)
			Oxidation of M (Mass;15.99)

Results and discussion:

1. A total of 1700 proteins were identified in the sample 1.
2. A total of 1830 proteins were identified in the sample 2.

*The list of proteins identified in each sample in the attached excel file.

Conclusion

- The attached excel sheet of the identified proteins requires filtration criteria including % of confidentiality depending on the researcher needs.

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