TEST REPORT

GENERAL DATA.

Test Report	**Preliminary**
Date	25/01/2023

Customer	**B.A.I. TECHNOLOGIES SA**
Request Analysis	2023/04-SV
Description of Samples	Sample of histological origin received on 12/20/2022; Analysis of interaction with Augmented NAC

SUMMARY

1. ACRONYMES

H_2 O: Deionized water.
ESI: Electrospray ionization.
MS: Mass Spectrometry.
CH_3 CN: Acetonitrile.
NH HCO_{43} : Ammonium bicarbonate.
HCOOH: Formic acid.

2. PURPOSE OF THE ACTIVITY

The aim of the analysis is to measure the degree of aggregation of the histologically derived protein complex in the presence and absence of Augmented NAC.

3. INSTRUMENTATION

Tool	Model	Manufacturer
Centrifuge	MIKRO 120	PBI
Freezer (-20 °C)	DV1	Evermed
Pipette	P20	Metler
Pipette	P100	Metler
Pipette	P1000	Metler
Mass spectrometer	LTQ	Thermofisher
Liquid chromatograph	Surveryor	Thermofisher
Thermoblock	DVV1	PBI

4. MATERIALS AND METHODS

4.1 Materials and reagents

4.1.1 Buffers used

Component	Manufacturer
Double distilled water	VWR
Acetonitrile (CH_3 CN)	VWR
Formic Acid (HCOOH)	VWR
Ammonium bicarbonate (NH HCO $)_{43}$	Sigma Aldrich

4.1.2 Reagents

Component	Manufacturer
Trypsin	Thermofisher

4.2 Sample preparation

4.2.1 Preparing Buffers

- Ammonium Bicarbonate (Digestion Buffer) 50 mmoL pH 7.8 **Preparation procedure:** You weigh, in a weighing pan, 0.4 g of ammonium bicarbonate (NH HCO_{43}). You pour the powder into a 100 ml bottle and add 100 ml (measured with the measuring cylinder) of double-distilled water. You shake the bottle until the NH HCO_{43} is completely dissolved.

4.2.2 Preparation of reagents

- Trypsin solution 12.5 ng/µL. **Preparation procedure:** You resuspend, in a vial, 20 µg of solid trypsin in 1600 µL of NH_4 HCO solution$_3$ 50 mM. One vortexes the vial until the trypsin is completely dissolved.

4.2.3 Enzymatic digestion

- A spatula tip of the sample was taken and placed in eppendorf sealed.
- Fifty µL of trypsin 12.5 ng/µL was added.
- Vortex for one minute.
- The sample thus treated was placed in thermoblock at 37° overweekend.
- It was centrifuged for 5 min at 13000g and the supernatant was transferred to vial.

4.3 LC-ESI-MS Instrumentation

The analysis was performed using an HPLC Surveyor (Thermofisher, USA). The column used was a Halo Peptide ES-C18, 2.1 x 50 mm, 2.7 µm. Analyses were performed using a two-phase gradient: Phase A (H_2 O+0.2 % Formic Acid (HCOOH)) and Phase C acetonitrile (CH_3 CN). The chromatographic gradient used is given in Table 1. The volume of sample injected is equal to 5 µL.

Data acquisition was done using a "SANIST" mass spectrometer. The ionization source used is an ESI.

Table 1**:** Chromatographic gradient used for the purpose of the feasibility study.

Gradient		
Time (minutes)	% C	Flow (mL/min)
0	2%	0.250
2.5	2%	0.250
3	80%	0.250
7	80%	0.250
8	2%	0.250
15	2%	0.250

4.4 Data Processing

Data processing was performed using SANIST Hb and Mascot packages.

5. RESULTS OBTAINED

Table 2 shows the degree of aggregation in percent of the complex detected in the absence and presence of Augmented NAC (2 µg/mg).

Table 2: List of samples analyzed

ID	Degree of aggregation % in **absence** of Augmented NAC	Degree of aggregation % in **presence** of Augmented NAC
1	89	32