

MOLECULAR SEROLOGY PROCEDURES MANUAL

Body Fluid Identification by Proteomic Mass Spectrometry - Liquid Chromatography & Mass Spectrometer Processing

Status: Published

Document ID: 77458

DATE EFFECTIVE 08/16/2023	APPROVED BY Molecular Serology Technical Leader	PAGE 1 OF 8
------------------------------	--	----------------

Body Fluid Identification by Proteomic Mass Spectrometry - Liquid Chromatography & Mass Spectrometer Processing

1 Purpose

- 1.1 Samples are run through liquid chromatography to separate peptide markers. Marker peptides are identified by mass spectrometry.

2 Check Before Starting

- 2.1 Check the LC/MS instrument to see how many batches are in the queue. **Do not proceed** if there are **two** batches in the queue. Check the queue again the next business day.
- 2.2 Check the Pure Air tank. If pressure on the large gauge (right gauge) is ≤ 300 psi, **do not proceed**. Contact the Proteomics Team to change the Pure Air tank if it is less than 300 psi.



- 2.3 Check all volumes in reagent bottles. If reagent bottles do not have the volumes listed below, **do not proceed**. Contact the Proteomics Team to change the reagents bottles if their volumes are less than the volumes listed below.

- 2.3.1 Methanol (50% methanol + 50% HPLC water) 200 mL
- 2.3.2 Loading **Solution** (phase A 2% ACN + 98% HPLC water) 200 mL
- 2.3.3 Bottle A (HPLC water) 160 mL

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Body Fluid Identification by Proteomic Mass Spectrometry - Liquid Chromatography & Mass Spectrometer Processing		
Status: Published		Document ID: 77458
DATE EFFECTIVE 08/16/2023	APPROVED BY Molecular Serology Technical Leader	PAGE 2 OF 8

2.3.4 **Bottle B (ACN) 120 mL**

2.4 For samples that need to be rerun, follow the instructions above (2.1 to 2.3 above) and then proceed to section 3.9.

3 Liquid Chromatography – Mass Spectrometer Analysis Procedure

3.1 Retrieve the following reagents:

Cytochrome C (1pmol/ μ l) at -80°C
PCM Standard
Phase A
Acetone
Acetonitrile
Isopropanol

3.2 If batch contains low concentration samples, make Low Conc resuspension mixture in a new 1.5 mL microcentrifuge tube. Work on ice or in a -20°C cold tube rack.

Low Conc. Resuspension Mixture – Enough for 9 Low Conc. Samples	
Reagent	Volume
Phase A	93 μ l
Cytochrome C (1 pmol/ μ l)	0.94 μ l
Total volume:	93.94 μ l

3.3 Resuspend samples as follows:

3.4 **REGULAR samples:** add 93 μ l Phase A and 0.94 μ l Cytochrome C (1 pmol/ μ l) to reconstitute peptides. **LIMS can do the math for total of samples**

3.5 **LOW Concentration Samples:** add 10 μ l of Low Conc resuspension mixture to reconstitute peptides.

3.6 Vortex.

3.7 Place in refrigerated centrifuge at 4°C and spin at 18,000 g for 30 minutes. Record instrument and temperature in LIMS.

3.8 Pipette supernatant into LC Vial (avoid pellet if present) and store at 4°C.

3.9 Prepare the LC cleaning solution to be run alongside samples and controls:

LC Cleaning Solution

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Status: Published		Document ID: 77458
DATE EFFECTIVE 08/16/2023	APPROVED BY Molecular Serology Technical Leader	PAGE 3 OF 8

Reagent	Volume
Acetone	2 µl
Acetonitrile	9 µl
Isopropanol	9 µl
Total Volume:	20 µl /sample

3.10 Place samples, controls, and prepared cleaning solution in Eksigent refrigerated auto sampler and fill out location of each vial in the input load plate on LIMS.

3.11 Fill in LIMS output data entry sheet and select the appropriate Acquisition Method for the sample type.

Sample Type	AcqMethod
PepCalMix, Phase A	Col1 PepCalMix Col2 PepCalMix
Cleaning Sol	Col1 Cleaning Col2 Cleaning
ENeg, Ext Pos controls, Unknowns	Col1 Unknown Col2 Unknown
Reruns (High)	Col1 Unknown Hi Col2 Unknown Hi

3.12 Output Plate Name should be the date (YYYYMMDD), followed by a letter identifying batch order (e.g. A, B, C, etc.) an underscore, followed by analyst initials.

3.13 Ensure the output samples are in the desired injection order before loading plate (in its default - vertical mode)

3.13.1 Positive controls should be run after unknown samples when creating batch.

3.14 Export sample batch from the output plate in LIMS.

3.15 Open LIMS created batch excel sheet in the LIMS LCMS folder and confirm it is as desired and save document as .txt format.



3.16 Open analyst software.

3.17 Double click Hardware Configuration → Eksigent LC and MS → Activate Profile

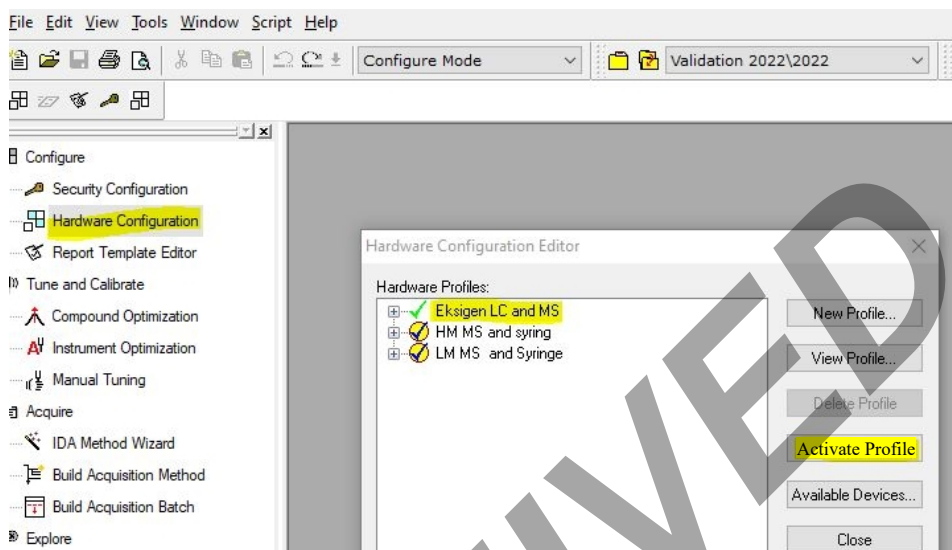
MOLECULAR SEROLOGY PROCEDURES MANUAL

Body Fluid Identification by Proteomic Mass Spectrometry - Liquid Chromatography & Mass Spectrometer Processing

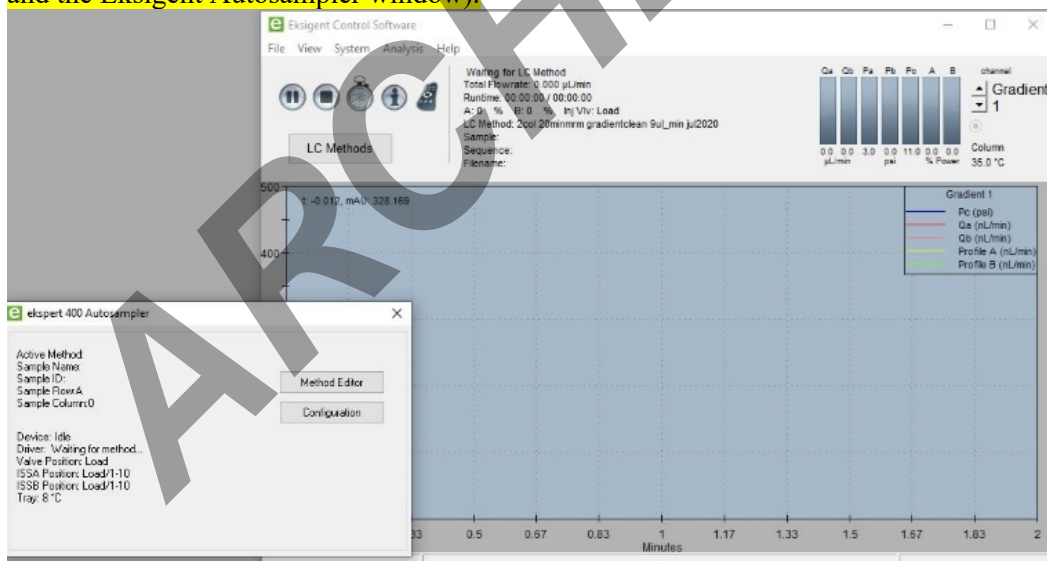
Status: Published

Document ID: 77458

DATE EFFECTIVE 08/16/2023	APPROVED BY Molecular Serology Technical Leader	PAGE 4 OF 8
------------------------------	--	----------------



- 3.18 Ensure that Eksigent windows is open simultaneously (both Eksigent Control Software window and the Eksigent Autosampler window).



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Document ID: 77458

DATE EFFECTIVE 08/16/2023	APPROVED BY Molecular Serology Technical Leader	PAGE 5 OF 8
------------------------------	--	----------------

3.19 Double click Build Acquisition Batch

The screenshot shows the Analyst software interface. The 'Acquire' menu is open, and 'Build Acquisition Batch' is circled in red. The main window displays a table of acquisition data with columns for Start Time, Sample Name, and Plate. A progress bar at the top indicates 100% completion for 20 samples.

	Start Time	Sample Name	Pla
1	6/14/2019 3:39:40 P	Phase A_Col2	1
2	6/14/2019 4:12:40 P	CytoC Dig_10fmol/ul 10ul>Loading_Col1	1
3	6/14/2019 4:45:41 P	CytoC Dig_10fmol/ul 10ul>Loading_Col2	1
4	6/14/2019 5:18:40 P	CytoC Dig_10fmol/ul 10ul>Loading_Col1	1
5	6/14/2019 5:51:41 P	Phase A_cbl2	1
6	6/14/2019 6:24:40 P	Phase A C1	1
7	6/14/2019 7:14:24 P	Phase A C2	1
8	6/14/2019 8:04:06 P	SE 0.1ug/ul, 3 ul loading, Col1_Sample 1	1
9	6/14/2019 8:53:49 P	SE 0.1ug/ul, 3 ul loading, Col1_Sample 1	1
10	6/14/2019 9:43:33 P	SE 0.1ug/ul, 3 ul loading, Col1_Sample 1	1
11	6/14/2019 10:33:14	SE 0.1ug/ul, 3 ul loading, Col1_Sample 1	1
12	6/14/2019 11:22:55	SE 0.1ug/ul, 3 ul loading, Col1_Sample10	1
13	6/15/2019 12:12:38	SE 0.1ug/ul, 3 ul loading, Col1_Sample 1	1
14	6/15/2019 1:02:20 A	SE 0.1ug/ul, 3 ul loading, Col1_Sample 1	1

3.20 Right click to import acquisition batch.

The screenshot shows the Analyst software interface. The 'Batch Editor' window is open, and the 'Import From' option in the context menu is circled in red. The context menu also shows 'File', 'SQL *LIMS Worklist', and 'SQL *LIMS TaskID'.

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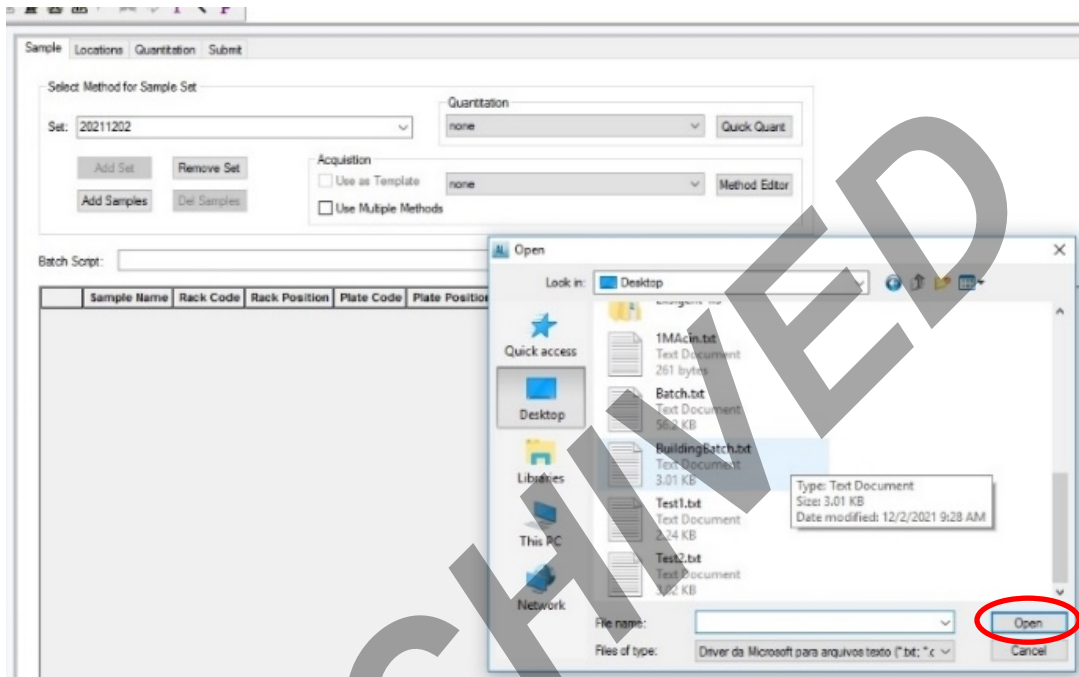
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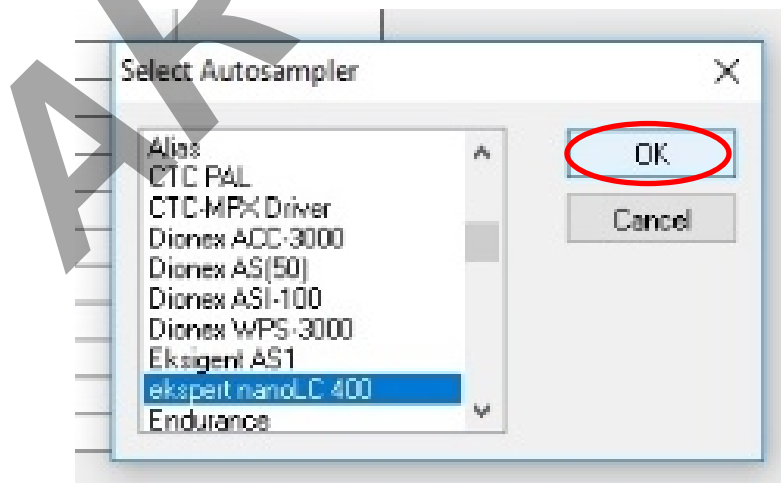
Document ID: 77458

DATE EFFECTIVE 08/16/2023	APPROVED BY Molecular Serology Technical Leader	PAGE 6 OF 8
------------------------------	--	----------------

3.21 Select .txt batch list exported from LIMS. Click open.



3.22 Select autosampler (ekspert nanoLC 400). Click OK.



3.23 **Label and Position WITNESS:** Have a witness verify the selected autosampler, batch sample names, methods, and tube positions in autosampler match that in Vial Position column.

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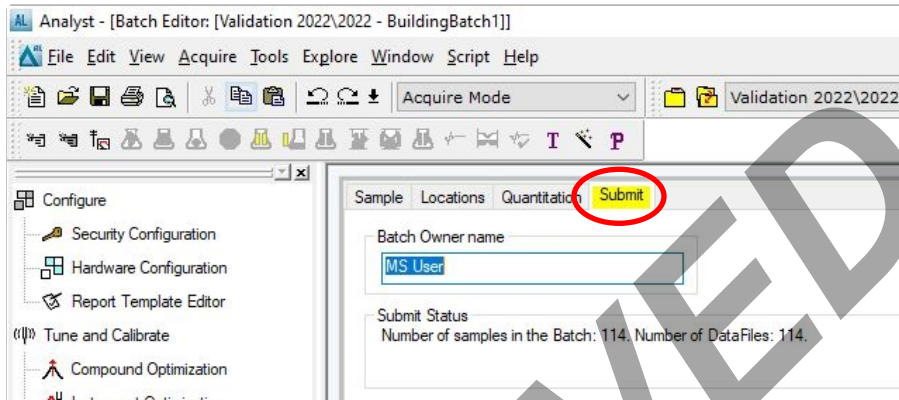
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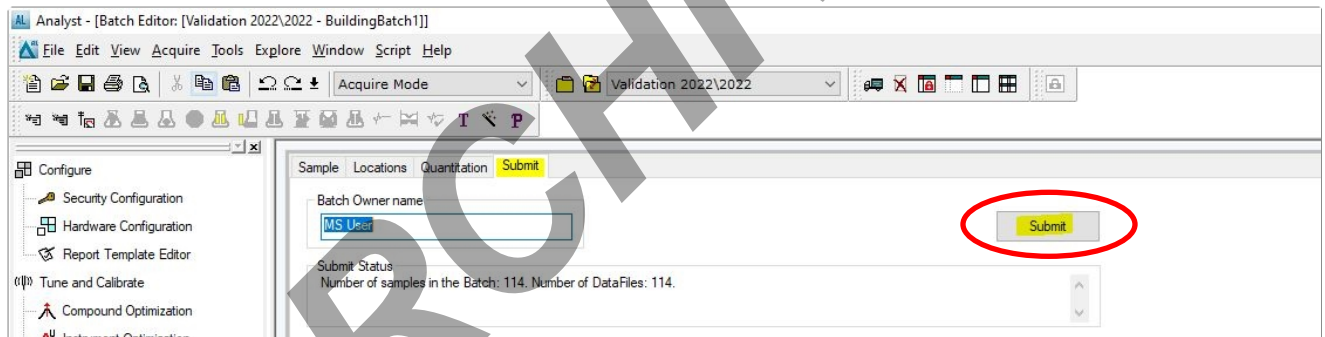
Document ID: 77458

DATE EFFECTIVE 08/16/2023	APPROVED BY Molecular Serology Technical Leader	PAGE 7 OF 8
------------------------------	--	----------------

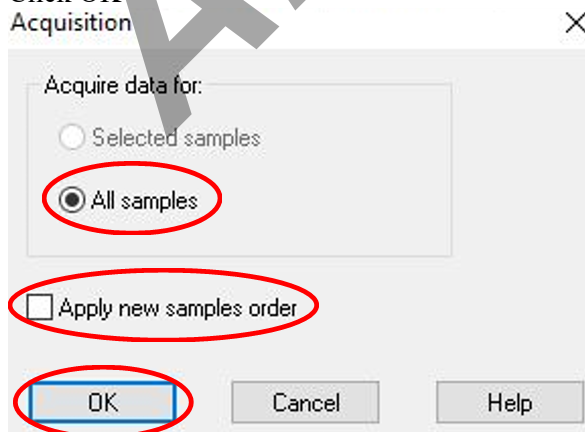
3.24 Click on Submit tab when sample list is ready.



3.25 Click Submit button.



3.26 Ensure "All samples" is selected and that the box for "apply new samples order" is unchecked. Click OK.



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DATE EFFECTIVE 08/16/2023	APPROVED BY Molecular Serology Technical Leader	PAGE 8 OF 8
------------------------------	--	----------------

3.27 Click Queue button and double check all samples were submitted.

The screenshot shows the Analyst software interface. The 'Queue' button in the toolbar is circled in red. The interface includes a menu bar (File, Edit, View, Acquire, Tools, Explore, Window, Script, Help), a toolbar with various icons, and a main panel with a progress bar and a table of sample data.

	Start Time	Sample Name	Plate Po	Vial Posi	Status	Method	Batch	Da
1	9/16/2022 2:17:52 P	001_20fmolul Pe	1	101	Waiting	2Col Run1grad2	New Batch	20

3.28 Click Ready button.

The screenshot shows the Analyst software interface. The 'Ready' button in the toolbar is circled in red. The interface includes a menu bar, a toolbar, and a main panel with a progress bar and a table of sample data.

	Start Time	Sample Name	Plate Po	Vial Posi	Status	Method	Batch	Data F
1	9/16/2022 2:17:52 P	001_20fmolul Pe	1	101	Waiting	2Col Run1grad2	New Batch	20220
2	9/16/2022 2:37:52 P	002_20fmolul Pe	1	101	Waiting	2Col Run2grad2	New Batch	20220

3.29 Click Start sample button. The Eksigent LC and 6500 MS will process all samples.

The screenshot shows the Analyst software interface. The 'Start sample' button in the toolbar is circled in red. The interface includes a menu bar, a toolbar, and a main panel with a progress bar and a table of sample data.

	Start Time	Sample Name	Plate Po	Vial Posi	Status	Method	Batch	Data F
1	9/16/2022 2:17:52 P	001_20fmolul Pe	1	101	Waiting	2Col Run1grad2	New Batch	20220
2	9/16/2022 2:37:52 P	002_20fmolul Pe	1	101	Waiting	2Col Run2grad2	New Batch	20220

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