Body Fluid Identification by Proteomic Mass Spectrometry - Liquid					
Chromatography & Mass Spectrometer Processing					
Status: Published Document ID: 774					
DATE EFFECTIVE	APPROVED BY	PAGE			
12/31/2024	Molecular Serology Technical Leader	1 OF 10			

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

Purpose 1

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 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.



- 2.2 Check the LC/MS instrument to see how many batches are in the queue. Ensure reagents are sufficient for all batches in queue. Assume all batches are full, i.e. consist of 82 runs.
 - 2.2.1 To calculate how much reagent volumes you need to proceed, count the number of batches ahead of you, add your batch and add 1 (safety batch) to the total. For example, if there are three batches ahead of you add: 3 (batches ahead of you) + 1 (your batch) + 1 (safety batch) = 5.
 - 2.2.2 From the table below you can calculate the volume of each reagent you need by multiplying the number of batches you calculated above (2.2.1) by the volumes of each reagent in the table. For example, above you would need 500 ml of 50% Methanol, 500 ml of Loading Solution A, 400 ml of Bottle A (HPLC water) and 300 ml of Bottle B (ACN).

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies. © NYC OFFICE OF CHIEF MEDICAL EXAMINER

Body Fluid Ch	Identification by Proteomic Mass Spec romatography & Mass Spectrometer Pr	trometry - Liquid ocessing		
Status: Published		Document ID: 77458		
DATE EFFECTIVE	APPROVED BY	PAGE		
12/31/2024	Molecular Serology Technical Leader	2 OF 10		
Now, check the the Proteomics	bottles. If you are unsure if there is enough volu Team.	me of a reagent, contact		
	TTTTTTTTTTTTT	11		

LC Pagant	Volume Needed /1 Full				
LC Keagent	Batch				
50% Methanol	<mark>100 mL</mark>				
Loading Solution Phase A	100 mL				
Bottle A (HPLC water)	<mark>80 mL</mark>				
Bottles B (ACN)	<mark>60 mL</mark>				

- 2.2.3 Check to see if there is a sufficient volume of PCM for your batch in the instrument tray.
- 2.2.4 To calculate how much reagent volume you need to proceed, count the number of batches ahead of you and add your batch to the total. For example, if there are three batches ahead of you add: 3 (batches ahead of you) + 1 (your batch) = 4.
- 2.2.5 Multiply the number of batches calculated above (2.2.4) by 18 μ l. For example, if you calculated four above, multiply by 18 μ l = 72 μ l.
- 2.2.6 To determine if there is enough PCM available, pipette the calculated volume using water into a new LC vial. Remove the PCM vial from the LC tray and compare it to the volume of water you pipetted into the new vial. If there is sufficient PCM in the PCM vial, replace the vial in the LC tray and proceed. If it is insufficient or you are unsure if there is enough PCM, contact the Proteomics Team.

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

Status: Published		Document ID: 77458
DATE EFFECTIVE	APPROVED BY	PAGE
12/31/2024	Molecular Serology Technical Leader	3 OF 10

2.3 Check Column and Tray temperatures as shown in figures below. Column temperature should be ~35°C and can be seen only on Gradient 1 window (shown below). Tray temperature should be \sim 8°C. If temperatures are not within \pm 2oC of recommended temperature, contact the Proteomics Group.



ekspert 400 Autosampler		×
Active Method: hyy 2ulpickup runcol2 2021june_0mm Sample Name:20240321A_QC_IA_002_Phase A Col2 Sample ID: Sample Row:L Sample Column:1	Method Editor	
Device: Idle Driver: Waiting for method Valve Position: Load ISSA Position: Load/1-10 ISSB Position: Load/1-10 Tray: 8 *C		

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies. © NYC OFFICE OF CHIEF MEDICAL EXAMINER

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

		occosing
Status: Published		Document ID: 77458
DATE EFFECTIVE	APPROVED BY	PAGE
12/31/2024	Molecular Serology Technical Leader	4 OF 10

2.4 For **samples that need to be rerun**, follow the instructions above (2.1 to 2.4 above) and then proceed to section 3.10.

3 Liquid Chromatography – Mass Spectrometer Analysis Procedure

3.1 Retrieve the following reagents:

Cytochrome C (8 pmol/ µl) at -80°C
PCM Standard
Phase A
Acetone
Acetonitrile
Isopropanol

- 3.2 Prepare a working solution of Cytochrome C Master Mix sufficient to resuspend all samples (see 3.3)Work on ice or in a -20°C cold tube rack.
- 3.3 Input into LIMS the number of regular quant samples + the number of low quant samples divided by eight. If the number is a fraction round up to the next integer. For example, if you have five regular quants and three low quant samples you have 5 + 3/8 = 5.38, therefore input 6 into LIMS.

Cytochrome C Master Mix Reagent Ratios (multiply volumes by the number of samples to be resuspended)			
Reagent	Volume		
Phase A	93 µl		
Cytochrome C (8 pmol/µl)	1.45 μl		
Total volume:	94.45 μl		

- 3.4 Resuspend samples as follows:
- 3.5 **REGULAR Samples**: add 94.45µl of Cytochrome C Master Mix (8 pmol/µl) to reconstitute peptides.
- 3.6 **LOW Concentration Samples**: add 10 µl of Cytochrome C Master Mix solution to reconstitute peptides.
- 3.7 Vortex.
- 3.8 Place in refrigerated centrifuge at 4°C and spin at 18,000 g (RCF) for 30 minutes. Record instrument and temperature in LIMS.

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

Status: Published	815 1	Document ID: 77458
DATE EFFECTIVE	APPROVED BY	PAGE
12/31/2024	Molecular Serology Technical Leader	5 OF 10

- 3.9 Pipette supernatant into LC Vial (avoid pellet if present) and store at 4°C.
- 3.10 Prepare the LC cleaning solution to be run alongside samples and controls:

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

- 3.11 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.
- 3.12 Fill in LIMS output data entry sheet and select the appropriate Acquistion 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

3.12.1 Your batch should not end on a "col 1" method. If your batch does end in a "col 1" method, add an additional phase A line at the end of the batch with a "col 2" method.

- 3.13 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.
- 3.14 Ensure the output samples are in the desired injection order before loading plate (in its default vertical mode)
 - 3.14.1 Positive controls should be run after unknown samples when creating batch.
- 3.15 Export sample batch from the output plate in LIMS.
- 3.16 Open LIMS created batch excel sheet in the LIMS LCMS folder and confirm it is as desired and save document as .txt format.

Body Fluid Identification by Proteomic Mass Spectrometry - Liquid Chromatography & Mass Spectrometer Processing Document ID: 77458 Status: Published DATE EFFECTIVE APPROVED BY PAGE 12/31/2024 Molecular Serology Technical Leader 6 OF 10



- 3.17 Open analyst software.
- 3.18 Double click Hardware Configuration \rightarrow Eksigent LC and MS \rightarrow Activate Profile



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

	Eksigent Control Software									- 0	×
	File View System Analysis H	Help									
	🕕 🗭 🏝 🛃	Walting Total Flo Runtime: A: 0 % LC Methe Sample: Sequence Filename	for LC Method wrate: 0.000 p 00:00:00 / 00: E: 0 % 1 od: 2col 20min ce:	uLimin 100:00 Inj Viv: Load mm gradientcle	xan 9ul_min jul2	2020		Qa Qb Pa 0.0 0.0 3.0 pL/min	Pb Pc A	B channel Gra 1 0.0 Column 35.0 °C	dient
	500 t -0.012, mAU: 328.169 400-									Gradient 1 Pc (ps) Ga (nL/min) Qb (nL/min) Profile A (nL Profile B (nL	Jmin) Amin)
ekspert 400 Autosampler	×										
Active Method. Sampb ID: Sampb ID: Sampb Ecourts Sampb Courter Device: Ide Driver: Wairing for method Vaive Ponter: Load 1158 Partient: Load 110	Method Editor Configuration										
Tray: 8 °C											
	33	0.5	0.67	0.83	1 Minutes	1.17	1.33	1.5	1.67	1.83	2

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies. © NYC OFFICE OF CHIEF MEDICAL EXAMINER Qualtrax template 040621

Body Fluid Identification by Proteomic Mass Spectrometry - Liquid Chromatography & Mass Spectrometer Processing Document ID: 77458 Status: Published DATE EFFECTIVE APPROVED BY PAGE 12/31/2024 Molecular Serology Technical Leader 7 OF 10

3.20 Double click Build Acquisition Batch

AL Analyst - [Queue Manager [Local]]					
K File Edit View Acquire Tools E	x <u>p</u> lore	<u>W</u> ind	dow <u>S</u> cript <u>H</u> elp		
🎦 🚅 🖬 🖨 🖪 X 🖻 💼 1			Acquire Mode	🔹 📑 🛃 Validation	
🍽 🍽 ta 👗 🕹 🕹 🕒 😃	8 7		🛃 👉 🖂 🍫 Т	×́Р	
Configure Security Configuration	A	quiri	ing Sample 20 of 20	D Period 1 of 1 Dur	ation xpecl
Hardware Configuration	0%		Start Time	Sample Name	Pla
Report Template Editor	1	\checkmark	6/14/2019 3:39:40 P	Phase A_Col2	1
((ψ)) Tune and Calibrate	2	\checkmark	6/14/2019 4:12:40 P	CytoC Dig_10fmol/ul 10ul Loading_Col1	1
Compound Optimization	3	\checkmark	6/14/2019 4:45:41 P	CytoC Dig_10fmol/ul 10ul Loading_Col2	1
	4	\checkmark	6/14/2019 5:18:40 P	CytoC Dig_10fmol/ul 10ul Loading_Col1	1
AI Instrument Optimization	5	\checkmark	6/14/2019 5:51:41 P	Phase A_col2	1
Manual Tuning	6	\checkmark	6/14/2019 6:24:40 P	Phase A C1	1
Acquire (2)	7	\checkmark	6/14/2019 7:14:24 P	Phase A C2	1
1 No. 10 Annual I	8	\checkmark	6/14/2019 8:04:06 P	SE 0.1ug/ul, 3 ul loading, Col1_Sample 1	1
	9	\checkmark	6/14/2019 8:53:49 P	SE 0.1ug/ul, 3 ul loading, Col1_Sample 1	1
E Build Acquisition Method	10	\checkmark	6/14/2019 9:43:33 P	SE 0.1ug/ul, 3 ul loading, Col1_Sample 1	1
Build Acquisition Batch	11	\checkmark	6/14/2019 10:33:14	SE 0.1ug/ul, 3 ul loading, Col1_Sample 1	1
	12	\checkmark	6/14/2019 11:22:55	SE 0.1ug/ul, 3 ul loading, Col1_Sample10	1
yer Explore (1)	13	\checkmark	6/15/2019 12:12:38	SE 0.1ug/ul, 3 ul loading, Col1_Sample 1	1
🛁 Open Data File	14	\checkmark	6/15/2019 1:02:20 A	SE 0.1ug/ul, 3 ul loading, Col1_Sample 1	1

3.21 Right click to import acquisition batch.



Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies. © NYC OFFICE OF CHIEF MEDICAL EXAMINER Qualtrax template 040621

Body Fluid Identification by Proteomic Mass Spectrometry - Liquid							
Status: Published Chromatography & Mass Spectrometer Processing Document ID: 77458							
DATE EFFECTIVE	APPROVED BY	PAGE					
12/31/2024	Molecular Serology Technical Leader	8 OF 10					

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

Γ

ple	Locations Quan	titation Submit						
Selec	ct Method for Sam	ple Set		Quantitation				
Set:	20211202		~	none	~	Quick Quant		
	Add Set	Remove Set	Acquisition		~	Mathed Editor		
	Add Samples	Del Samples	Use Multiple Metho	ds		Horios Estar		
	Ca.44			M Open				
ten :	script.			Lock i	Desktop		- G 🕸 📂 🖬 -	
_	Sample Nam	e Rack Code Rack	Position Plate Code Plat	e Position	() energen			
	Sample Nam	e Rack Code Rack	Position Plate Code Plat	e Position Quick access Desktop	1MAcin.t Text Doc: 261 bytes Batch.txt Text Doc: 56.2 KB	at ument ument		
	Sample Nam	e Rack Code Rack	Position Plate Code Plat	a Position Quick access Desktop Libraries	1MAcin.t Text Doc 261 bytes Batch.bat 56.2 KB Building Toxt Doc 3.01 KB	at ument Batch.txt ument	pe: Text Document	
	Sample Nam	e Rack Code Rack	Position Plate Code Plat	e Position Quick access Desktop Libraries This PC	1MAcin.t Text Doc 261 bytes Batch.sd 56.2 KB Building Toxt Doc 56.2 KB Building Toxt Doc 3.01 KB Text Loc 2.24 KB	at ument Satch.bt ument Siz ument Da	pe: Text Document er 3.01 KB te modified: 12/2/2021 9:28 AM	
	Sample Nam	e Rack Code Rack	Position Plate Code Plat	a Position Quick access Desktop Libraries This PC	1MAcin.1 Text Docy 261 bytes Batch.3d Building Text Docy 56.2 KB Building Text Docy 3.01 KB Text Docy 3.01 KB Text Docy 2.24 KB Text Docy 3.02 KB	at ument Satch.bst ument Da ument	pe: Text Document te: 3.01 KB te modified: 12/2/2021 9:28 AM	

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

		,
Alias CTC PAL CTC-MPX Driver	^	OK Cancel
Dionex ACC-3000 Dionex AS(50)	_	
Dionex ASI-100 Dionex WPS-3000		
Eksigent AS1		
Endurance	- v	

3.24 **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.

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies. © NYC OFFICE OF CHIEF MEDICAL EXAMINER Qualtrax template 040621

Body Fluid Identification by Proteomic Mass Spectrometry - Liquid Chromatography & Mass Spectrometer Processing Document ID: 77458 Status: Published DATE EFFECTIVE APPROVED BY PAGE 12/31/2024 Molecular Serology Technical Leader 9 OF 10

3.25 Click on Submit tab when sample list is ready.



3.26 Click Submit button.

Analyst - [Batch Editor: [Validation 202	2\2022 - BuildingBatch1]]
Eile Edit View Acquire Tools Exp	<u>p</u> lore <u>W</u> indow <u>S</u> cript <u>H</u> elp
12 🖙 🖬 🖨 🖪 🔺 🖪 🛍 🖆	2 🕰 🛨 🗛 Acquire Mode 💎 🖆 🖓 Validation 2022/2022 🗸 😝 🕅 🕅 🗂 🖽 🖪
🛏 🗤 ta 🕹 🛎 🕹 🖷 🚨 🛀	L Y M L T K F
E Configure	Sample Locations Quantitation Submit
Security Configuration Hardware Configuration Report Template Editor	Batch Owner name MS User Submt
((Ų)) Tune and Calibrate → Compound Optimization	Submit Status Number of samples in the Batch: 114. Number of DataFiles: 114.

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

Selected samples All samples	 Selected samples All samples Apply new samples order 	Acquire data for:	
All samples	All samples	O Selected samples	
] Apply new samples order	All samples	
	Apply new samples order		
		Apply new samples order	

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies. © NYC OFFICE OF CHIEF MEDICAL EXAMINER

Body Fluid Identification by Proteomic Mass Spectrometry - Liquid Chromatography & Mass Spectrometer Processing Status: Published Document ID: 77458 APPROVED BY DATE EFFECTIVE PAGE 12/31/2024 Molecular Serology Technical Leader 10 OF 10

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

Analyst - [Queue Manager [Local]]									
Tile Edit View Acquire Tools Exp	lore <u>W</u> indo	w <u>S</u> cript <u>H</u> elp							
🎦 🖨 🖶 🎒 🖪 🕹 🖺 의	C* ± Ac	quire Mode	🗸 🗖 🔁 v	alidation 2	2022\2022	: •	- 🕫 🗙 🛅 🗖		
🔫 🕫 🕹 🛎 🕹 🔍 💷 🗉	I W M J	l 👉 🖂 🍫 T 🤻	P						
View Queue	Acquir	ing Sample 0 of 0	Period 0	of 0	[Durations Expected	00:00:00	Queue Server	
Security Configuration	0%				100%	Elapsed	Stand	IBy Norma	
Hardware Configuration		Start Time	Sample Name	Plate Po	Vial Posi	Status	Method	Batch	Da
Report Template Editor	1 🗵	9/16/2022 2:17:52 P	001 20fmolul Pe	1	101	Waiting	2Col Run1grad2	New Batch	20

3.29 Click Ready button.

Analyst - [Queue Manager [Local]]									
Eile Edit View Acquire Tools Expl	lore <u>W</u> ind	low <u>S</u> cript <u>H</u> elp							
🖹 🖨 🖶 🖨 🗟 🐰 🖻 💼 🗠 🕰 Ł Acquire Mode 🖓 Validation 2022\2022									
14 14 ta & & & & 🗛 🛄 🛛	a a a	🕭 🖛 🛱 🤝 Т 🔍	P						
Configure Security Configuration	Acc 0%	uiring Sample 0 Ready	Period 0	of 0	100%	Durations Expected Elapsed	00:00:00 🔀	Queue Server 말물 I By Norma	1
		Start Time	Sample Name	Plate Po	Vial Posi	Status	Method	Batch	Data F
······ 🐼 Report Template Editor	1	9/16/2022 2:17:52 P	001_20fmolul Pe	1	101	Waiting	2Col Run1grad2	New Batch	20220
((Ųi)) Tune and Calibrate	2	9/16/2022 2:37:52 P	002_20fmolul Pe	1	101	Waiting	2Col Run2grad2	New Batch	20220

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

Analyst - [Queue Manager [Local]]									
K Eile Edit View Acquire Tools Explore Window Script Help									
🖹 🖨 🖶 👰 🖪 🐇 🖻 🖹 🗠 🕰 Ł Acquire Mode 💎 📫 🔂 Validation 2022\2022 🗸 🗐 🕮 🕅 🗔 🖽 🔴									
🛥 🗃 🕈 🛦 🖉 🖉 🖉 🖉		🕭 👉 🖂 🍫 Т 🚿	P						
Configure	Acqu	iring Sample 0 Ready	Period 0	of 0	1000	Durations Expected	00:00:00	Queue Server	
Hardware Configuration	0%	Start Time	Cample Name	Dista Da	Viel Deei	Statua	Stand	1 By Norma	Data
Template Editor	1 5	9/16/2022 2:17:52 P	001_20fmolul Pe	1	101	Waiting	2Col Run1grad2	New Batch	20220
«ψ» Tune and Calibrate	2	9/16/2022 2:37:52 P	002_20fmolul Pe	1	101	Waiting	2Col Run2grad2	New Batch	20220

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies. © NYC OFFICE OF CHIEF MEDICAL EXAMINER Qualtrax template 040621