

MANUAL proteoCHIP LF 48

for the Label Free Single Cell Proteomics workflow using the cellenONE®



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1. Introduction

The cellenONE software monitors cells inside the piezo dispensing capillary before dispensing. All steps associated with single cell isolation and dispensing experiments are presented in the Software User Guide.

Note: It is higly recommended to read the Software User Guide before performing any experiment with the cellenONE.

This Manual will guide you through all steps of the workflow dedicated to single cell proteomics label-free experiments using proteoCHIP LF 48.

IBSCI™: Image-Based Single Cell Isolation

IBSCI[™] is a high resolution-based technology consisting of automated image acquisition, processing and advanced algorithms to automatically isolate single cells from a cell suspension.

FIBSCI™: Fluorescence Image-Based Single Cell Isolation

FIBSCI[™] utilizes multichannel fluorescence-based images to identify and isolate single cells of interest from subpopulations in mixed cell samples based on the presence, absence and intensity of a fluorescent signal.



2. Materials

Part number	Description	
P-20-CL	cellenONE PDC L Piezo Dispensing Capillary(Fixed drop volume between 450-600 pl)4x manifold	
P-20-CM	cellenONE PDC M Piezo Dispensing Capillary (Fixed drop volume between 300-450 pl) 4x manifold	
C-PLF-48-3	 proteoCHIP_LF_48 The proteoCHIP LF 48 Set containing: 3 (three) proteoCHIP LF 48 	A REAL PROPERTY OF A REAL PROPER
	The proteoCHIP LF 48 is a pyramidal-shaped bottom nanowell chip, 48 nanowells, prefilled with oil, enabling label-free single cell proteomics sample preparation.	
	proteoCHIP_LF_48 cellenONE Holder Holder to accommodate up to 3 proteoCHIP_LF_48 on the deck of the cellenONE throughout sample preparation (cell isolation, reagent dispensing and incubation steps). (1 piece/pack)	
C-PLF-48- CHT	proteoCHIP_LF_48 cellenONE Holder Top Top to maintain the proteoCHIP_LF_48 inside the cellenONE Holder on the deck of the cellenONE throughout sample preparation (cell isolation, reagent dispensing and incubation steps). (<i>1 piece/pack</i>)	



3. cellenONE setup

3.1. Nozzle setup and Drop parameters setup

Before starting your experiment, you will need to make sure that your PDC is correctly positioned and produces stable droplets.

At the beginning of the priming, the PDC should be centred with the red cross. You can align your PDC using:

Nozzle Setup >> Nozzle Offset >> Up, Down, Back, Front, Left, Right controls (Figure 1)

After the priming enter the Voltage and Pulse parameters (indicated on the PDC box), then click on:

- Set Nozzle Parameters
- Start Continuous Dispensing

If needed, adjust the Voltage and Pulse until you have a stable drop and then press:

Set Nozzle Parameters



Figure 1. Drop optimisation

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3.2. Humidity and temperature setup

Throughout the workflow, the humidity and temperature need to be set to a specific value to avoid evaporation and minimize condensation.

Note. The runs already contain specific tasks controlling humidity and temperature for each run, but you should verify that the dew point temperature (DP) is above the temperature in the enclosure (BT) to avoid evaporation (BT<DP). We recommend setting -1 °C dew point correction (see Figure 4).

The following describes how to change humidity/temperature "manually" if you wish to change it.

• Humidity setup

Main >> File >> Humidity Control >> Popup window >> Set humidity >> Click on "Set" Figure 2).



Figure 2. Humidity control

• Temperature setup

Main >> File >> Cooling Unit Control >> Popup window >> Set the temperature to a fixed control or at Dew point and turn the cooling unit ON.

Please also make sure that the Dew Point Correction Value (Cooling Unit Control >> Popup window >> Settings tab) is set to -1 (Figure 4).





Figure 3. Temperature control

🗘 Cooling Unit	×
Cooling Unit Settings	
Bath Port	Cooling Unit 0FF
Pump Speed 2000	Dew Point Control
Dew Point Correction Value	Temperature 0
	Create Task

Figure 4: Dew Point Correction Value

Note. During a long cell isolation run, the -1 °C Dew Point Correction Value can result in some condensation on the plate. However, this is not affecting the Master Mix efficiency neither is it leading to cross contamination.

Note. When doing a label free experiment, it is best to start by setting the temperature to 10 °C.

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3.3. Target setup

Note. To teach the proteoCHIP_LF_48, please refer to the "proteoCHIP_User Manual_proteoCHIP_LF_48_Teaching" manual.

To display your target, go to:

• Target Setup >> Target

For the proteoCHIP LF, you will always have the following configuration in Target Setup:

- Start point: 0, 0
- Field Gap: 0,0
- Number of Fields: X=1, Y=1
- Spot per field: X=12, Y=4
- Spot pitch: X=4500 μm, Y=4500 μm

🗘 Main File Target	Field Ta	ask List H	elp						
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Target	Field Se	tup							
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Field Na Selecte Target Im	ime ed Field age _X 0	1	γO			All	5c 1.0	ale	1.5 2.0 2.5 3.0
Field 1									
			•				•		
		•				• • • •			

Figure 5. Target setup



3.4. Field setup

The "Field Setup" sub-tab is used to define:

- From which well the sample will be aspirated.
 - → ONLY if the run contains a "TakeProbe" task (This is the case in the LF protocol.).
- Number of drops/volume to be dispensed in which "spot" of the Target.

Note. To change from Volume to drops and vice versa:

• Target Setup >> Field Setup >> "Field" at the top >> Volume entry

Note. For every run, fields are saved in the software.

You can edit these Fields using "Edit Field Table" to change:

- From which well the sample will be aspirated.
- Number of drops/volume to be dispensed in which "spot" of the Target.

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		*****	1/4	1A	1,	1,	4 0 0000000000
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			1/6	i 1A	1,	1,	
			• • 1/7	1A	1,	1,	
			1/8	1A	1,	1,	
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			1/1	1 1A	1,	1,	
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		121	3/3	1A	1,	1,	
	Wall Order	Dauce Cet	tinne 3/4	14	1.	1.	

Figure 6. Field setup

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Note. To assure that the exact suggested volume of each reagent is dispensed we recommend performing a Drop volume to calculate the corresponding number of drops before each liquid dispensing run.

This should be done before dispensing the Master Mix, H_20 in the rehydration steps and 0.1% TFA/FA.

To modify the number of drops/volume in the Field setup refer to Figure 7 and change the number of drops/volume by clicking on the Drops subtab.



Figure 7. Edit Field Table

3.5. Drop Setup & Start Dispensing

Each dispensing run is built with the same logic, once the run starts (Figure 8):

Several pop-up messages will prompt you to load the correct volume of the desired solution in the well specified in the Target Setup section.

- The sample should be loaded in a chosen well of the 384 source plate (The well is chosen in the Field Setup tab, see paragraph 3.4).
- The volume to be aspirated is predefined inside the Run by the "TakeProbe" task. (*Required volume will be mentioned in the message that will pop up at the beginning of the run*).

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- The capillary (PDC) moves to the chosen well, aspirates the chosen volume and moves back to do a wash cycle and take a picture of the drop.
- The software will automatically check that the dispensing parameters are correct by performing a "DropCheck". Should the system fail to detect a suitable drop, the run will pause and the user will be able to modify the parameters to restore a stable droplet generation.
- The user must check that the dispensing parameters (Voltage and Pulse) (Figure 8 (1)) are correct by pressing the "Continuous Dispensing" button (Figure 8 (2)) until a drop is stable without any satellite (if any).
- The user must save the new settings if the Voltage and Pulse were modified (Figure 8 (3)) and press the "Drop Check" button (Figure 8 (4)) and check if the drop is identified by the software (circled in red) (Figure 8 (5)).
- If needed, you can also perform troubleshooting tasks (AirEx, Flush_PDC, etc.) and uptake more sample.
- Once the droplet generation is stabilized, click "Continue" to resume the run (Figure 8 (1)).



Figure 8. Drop Setup and start dispensing



3.6. proteoCHIPs inside the cellenONE



Figure 9. proteoCHIP LF inside the cellenONE holder

 Place the proteoCHIP(s)_LF_48 inside the cellenONE holder (ref. C-PLF-48-CHB) (Figure 9).

Note. If processing only one chip, insert it on the spot closest to you.

• Place the cellenONE holder top (ref. C-PLF-48-CHT) on top of the holder and insert the assembled unit on the target holder inside the instrument.

Note. The experiment is a standardized workflow for only one proteoCHIP LF 48. If you are doing more than one proteoCHIP LF 48 in parallel, you will need to repeat run 5 for each proteCHIP LF 48.

e.g., If you are running 3 proteoCHIPS you will need to do:

1X the run 1 1X the run 2 1X the run 3 1X the run 4 3X the run 5



4. Step 1: Master Mix dispensing

Note.

We recommend using this Master Mix (Lysis + Digestion): **0.2% DDM, 10 ng/μL Enzyme(s), 100 mM TEAB** For each experiment 100 μL of Master Mix is needed.

To prepare 100 μ L of Master Mix add the reagents in the following order: 10 μ L 1M TEAB + 60 μ L H₂O + 20 μ L 1% DDM + 10 μ L 100 ng/ μ L Enzyme(s)

Do not vortex the mixture, this will introduce air bubbles. Mix by gently pipetting the solution up and down.

Tip: Aliquot 20 μ L 1% DDM and 10 μ L 100 ng/ μ L Enzyme(s) at -20 °C for short term usage. For long term storage of enzymes, we recommend storage at -80 °C. TEAB should be stored at 4 °C.

Note.

We recommend using MS-grade enzymes (e.g., Trypsin Gold, Promega or Trypsin/LysC Mix, Thermo). For enzyme mixtures we recommend to also use a 10 ng/ μ L mixture of the enzymes.

Note.

For the Master Mix, you will probably need higher Voltage (+5-10%) to achieve an optimal drop formation. You can also optimize that during the run by clicking Nozzle Setup.

4.1. Probe, Run and Target selection

The Master Mix dispensing happens at 8 (+/- 2) °C and at humidity set to 45%.

In the Main tab of the software, select the probe, run and target to be used (Figure 10).

- Probe: "MTP384"
- Run "LF_48_Run_1_MasterMix"
- Target: "proteoCHIP_LF_48"

This run contains an intermediate task that checks the stability of the drop after the dispensing of each row of the proteoCHIP (Figure 15). If the drop is not stable, you will have the opportunity to optimize the parameters, as explained in paragraph 3.5.





Figure 10. Run 1 Master mix dispensing

4.2. Field selection

Load the field "LF_48_Run_1_MasterMix" which will spot 400 drops twice (~300 nL) per well.

Note.

```
As the run will dispense the Master Mix in two rounds, to have the correct end volume (~300 nL), you need to modify the field in volume entry to dispense 150 nl.
```

• To load the field:

"Target Setup" >> "Target" >> "Load Field" >> Select the field corresponding to the run (Figure 11) and you will have the field corresponding to Figure 12.



Contractions of the second sec	in arget Field Task List Help Coon Target Selection Window Lood Field Save Field Copy Field Solution Field Offset Sort by Field Position Line Spotting Line Spott	Run Robot S Ho. of Fields X 1 v Y 1 w Idrop.fid A	ietup H [%]: 52 Spot per Field X 12 V 4 0 1.0	T [*C] 22	Auto Center Auto Center X OFF/ON 1.5 2.0 2.5 3.0	2neles
3 🗾	me ^		C g	Date modified 1/29/2022 5:23 Pl	Type M FLD File	
	LF_48_Run_2_Cells LF_48_Run_3&4_Incubation& LF_48_Run_5_Dilution	ጵCooldown	7 9 9	//8/2022 3:30 PM //29/2022 5:24 PI //21/2022 3:55 PI	I FLD File M FLD File M FLD File	

Figure 11. Load Field for MasterMix dispensing



Figure 12. Field Run 1



4.3. Start the Run

Note. Load 60 μ L of the Master Mix in the selected well.

Once the proteoCHIPs are loaded in the cellenONE as seen in Figure 9, check that you selected the right probe, run and target in the run window (Figure 13(1)). Start the run (Figure 13(2)), name the experiment (Figure 13(3) and click OK (Figure 13(4)). Select the number of proteoCHIPs that you want to dispense (Figure 13(5)) and click "OK" (Figure 13(6)).

A few pop-up messages will guide you before starting to spot (Figure 14).

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2 Start Run L 48_Run_1_MasterHix Target proteoCHIP_LF_48 End Name flast loaded or source)		proteoCHIP 3
LF_48_Run_1_MasterNor.fd LF_48_Run_1_MasterNor.fd Lf_48_Run_1_MasterNor.fd Start Time: Start Time: Estimated Completion	Sort By X Field Position Time: Hultidrops	proteoCHIP 2
Plate No. Well Nozzle Drops Target No. Field	eo. X Pos. Y Pos. 4 Auto Drops to Discard \$ 5	proteoCHIP 1

Figure 13. Starting the MasterMix dispensing run

⑦ Dialogue ×	Comment	×	Comment ×	Comment ×
Please, insert MTP384 probe and press "OK".	The system will now cool down to 20 C. Once this temperature is reached, you may start Run 5. This will take about 30 minutes.	^	Before pressing OK, please make sure that the A oil is completely frozen.	Load 60 µL of Master Mix in the source plate - A make sure that the proper target actup is loaded - Then press CONTINUE
		~	~	~
OK Abort	OK		ОК	CONTINUE

Figure 14. Pop-up messages

Note. ALWAYS use at least the target 1 so the "AutoDropDetection" and "OpenNozzleSetup" works properly (first proteoCHIP as shown above).

Target Field Task	List Help		
		H [%]: 51 T [°C] 22	cellenONE mode User logg
	Probe MTP384		Live Camera
Start Run	Run Run_1_MasterMix_300nL_1chip Target proteoCHIP_LF	Stop Nozzle Setup	∧ Nozzle Image − × Second (m/k): 23. Deviation (m/k): 14.
Start Time: 3:57 P	Field Name (last loaded or saved) Run 1_MasterMix_1_48wells_750drops_Chip re Nozzle Offset Parallel Spotting 1 - 7/8/2022 Estimated Comp	1.fld	
Plate No. Well	Nozzle Drops Target No.	Field No. X Pos. Y Pos.	0
Comment			

Figure 15. Autodrop check



5. Step 2: Cell isolation

Prepare your cells at a concentration of 100-200 cells/µL in degassed PBS.

5.1. Probe, Run and Target selection

In the Main tab of the software, select the probe, run and target to be used (Figure 16).

- Probe: "MTP384"
- Run "LF_48_Run_1_MasterMix"
- Target: "proteoCHIP_LF_48"

ist Help							
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Figure 16. Run 2 Single cell isolation

5.2. Field Selection

Click on the 3rd tab, "Target Setup", and the 1st sub-tab, "Target". Target is the same for the whole workflow, but the field setup will need to be changed at every step.

Note. To isolate and dispense single cells, positions defined in the field setup must contain only one drop per well.

Load the field "LF_48_Run_2_Cells" which will spot 1 drop (=1 cell) per well. To load the field, click in "Target" (Figure 17) >> "Load Field" (Figure 17), select the field corresponding to the run (Figure 17) and you will have the field corresponding to the Figure 18.



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Figure 17. Load Field for cell isolation



Figure 18. Field Setup for cell isolation

Note. If you wish to dispense more than 1 single cell, the following syntax must be used: 1, 1 (for isolation of two single cells); 1, 1, 1 (for isolation of three single cells); 1,1,1,1 (for isolation of four single cells), etc. You can do so by clicking multiple times (n) on the well where you want to have 1+n cells isolated.



5.3. cellenONE module

Note. Load > 30 μ L of cell suspension (100-200 cells/ μ L) into a chosen well of the 384-well probe plate.

For this run you will need to **open the cellenONE window** and start the run in this same window. Once the **cellenONE module** is opened, it **should not be closed** before the end of the run (nevertheless the window can be minimized).

Please refer to the Software Reference Guide Chapter 7 "Single cell isolation" to understand all the options and features for single cell dispensing.

Select the "Nozzle Setup" tab (Figure 19 (1)) and press on the cellenONE logo (Figure 19) to open the cellenONE module for single cell isolation.



The cell isolation run is still performed at 10 °C and ~45% humidity.

Figure 19. cellenONE module for single cell isolation (right window)

Before starting, define the folder location and name your experiment by pressing the following



All files associated with your experiment will be saved in this location. By default, the data is saved in the last folder chosen and named samplename_date.

Start the run by clicking on the





. You will

Please note that you can always pause the run with the Nozzle setup shortcut be able to modify the parameters if wanted.

Select the proteoCHIPs that you want to dispense in (Figure 20) and click "OK" (Figure 20(4)).

Directory for Run Folders		Set Hodde On Al On 1 0 OF Al OF Exercic 2.4	Add Copy Hobie Target Seake OFF F11 Rev No. 1 to al mobiles Shortest Path OFF	0
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Volume limit Sample Volum	me [µl] 10 📮	I		
Save Images Isolated 🗸	Enrichment		ProteoCHIP	2
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OF	Cancel		ProteoCHIP	T

Figure 20. Starting the run for single cell isolation

1	🗘 Dialogue	×	Comment	×	Comment	×
	Please, insert MTP384 probe and press "OK". WARNING - The PDC is going to uptake. Please do not open the door. Risk of PDC breakage!	^	Set up cell isolation parameters. When done press CONTINUE to resume isolation.	^	Load 30 µL of cell suspension (100-200 cells/ µL) in the well specified in target setup - Then press CONTINUE.	^
	OK Abort	v	ОК	×	CONTINUE	~

Figure 21. Pop-up messages

After uptaking the desired volume of the cell sample, the PDC comes back to the camera station so you can define the cell parameters used for cell isolation.

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5.3.1. Setting the detection parameters

The "Main" tab of cellenONE module (Figure 22) displays images of the region of interest of the PDC in which cells are detected. During dispensing, cells are travelling from right (upper part of the nozzle) to left (nozzle tip). Information below the image (i.e., Diameter, Elongation, Circularity, and Intensity) is given for the leftmost detected cell, i.e., the closest to the nozzle tip.



Figure 22. Main tab of cellenONE® module.

Hint. The elongation factor can be used to effectively differentiate two aggregated cells from a single cell as these will have different elongation factors.



This section is used to define detection and isolation parameters (Figure 23). These parameters are crucial to successfully isolate and dispense single cells.



Figure 23. Detection and isolation parameters

Detection parameters: These parameters will determine what the software will detect and consider as an object (e.g., cell or particle). As a result, these parameters should be "large" enough to ensure that all cells and cell clusters are detected as objects.

- **Min diameter:** detected diameter of the smallest object that should be detected. An object with a diameter lower than the entered value will **not be detected**.
- Max diameter: detected diameter of the largest object that should be detected. Beware that cell aggregates will typically be detected as a single large object. An object with a diameter bigger than the entered value will **not be detected**.
- Elongation: an object with an elongation above (for MAX) or below (for MIN) the entered value will **not be detected**.
- *HINT 1* The detected diameter is typically about 10% larger than real diameter.

HINT 2 A detected object is surrounded by a green or a red circle:

- a green circle means that the object fulfils the isolation parameters and would be isolated.
- a **red circle** means that the object does not fulfil the isolation parameters and would not be isolated.

HINT 3 To start, set wide detection parameters and then, restrict them if needed.

- Diameter min: 5 µm
- Diameter max: 100 µm
- MAX Elongation: 4

After loading the sample, do a background:



Get background button: Takes a reference background image. This button should only be pressed when the nozzle is free of cells and/or particles and when the PDC is in front of the camera.



Make several drops:



Test droplet button: Ejects a single droplet.

Check visually by eye that every particle inside the PDC is detected (i.e., that the particle is circled by a red or green circle).

5.3.2. Setting the ejection boundary

Once detection parameters are set, do a mapping:



Start mapping button: Starts the mapping process.

This process maps the position of cells inside the PDC during the dispensing of droplets in front of the camera. This will determine **the size of the ejection zone** (Zone 1 on Figure 22). Pressing this button during mapping will interrupt the mapping process.

At the end of the mapping process, the ejection boundary is set automatically according to the blue and green dot distribution inside the PDC (Figure 24). Cells that were detected in the location of blue dots were subsequently detected again inside the PDC upon next droplet generation. Whereas green dots correspond to the last seen location of a cells within the PDC (i.e., upon next droplet generation these cells were ejected from the PDC). Once done, click "Save & close".



Figure 24. Mapping

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Hint. At the end of the mapping process, the ejection boundary is set automatically according to the distribution of the blue and green dots inside the PDC. To **adjust the ejection zone manually, move the slider** by clicking and dragging the positioner on the ruler below the image and **press "Set" to validate**.

5.3.3. Setting the isolation parameters

Once Mapping is done, set up your isolation parameters.

Isolation parameters: These parameters can be determined from the characteristics of the cells shown in the mapping report (Figure 24) and adjusted as needed to include only cells of interest. These values are critical to ensure satisfactory single cell isolation. All objects that fulfil the isolation parameters and single cell condition will be isolated.

- Min diameter: detected diameter of the smallest object that should be isolated.
- Max diameter: detected diameter of the largest object that should be isolated.
- Elongation: an object with an elongation above (for MAX) or below (for MIN) the entered value will not be isolated.

Three options are available here:

- Draw a gate right after the mapping from the scatter plot that was just performed or enter values manually into the isolation parameters based on the mapping.
- Open the mapping (in the folder you chose earlier, folder name.mapping) and interpret the different scatter plots.
- Perform an analysis of your sample.



Analyze sample button: Analyzes 100 cells from your sample.

This can be used to estimate the heterogeneity of your sample. At the end of the analysis, a window will pop up with scatter plots (Figure 25). The number of analysed cells can be set in the "Advanced Settings" tab. You can do a gate to choose new values for isolation parameters.



Figure 25. Scatter Plot after Analysis



5.3.4. Checking the setup of your experiment

Check the ejection boundary:



Test droplet button: Press it manually multiple times to verify the ejection boundary. When a particle is inside the ejection zone, it should be ejected within the next droplet when pressing the droplet button once more.

Check the isolation parameters:

cells (or particles) of interest should be circled with a green circle and the other particles with a red circle.

Note. Continuous drop generation can be activated by simultaneously clicking on the left mouse button and Shift button and is interrupted by clicking once again on the test droplet button.

Once you are satisfied with your parameters you can click Continue to start dispensing your cells (Figure 26).



Figure 26. Starting cell isolation Run

6. Step 3: Incubation at 50 °C

Single cells have been isolated in wells containing lysis and digestion buffer. The next step is to perform the incubation to:

- Lyse the cells,
- Digest the protein into peptide fragments.

This step can be performed directly on the deck of the cellenONE. This incubation step will last 1h30, at a temperature of 50 °C. In addition to an increased ambient humidity, the PDC will regularly dispense deionized water in each well to maintain a constant water content and create a mechanical mixing phenomenon to help achieving homogeneous cell lysis and protein digestion.



6.1. Probe, Run and Target selection

In the Main tab of the software, select the probe, run and target to be used (Figure 27).

- Probe: "MTP384"
- Run "LF_48_Run_3_Incubation_50C"
- Target: "proteoCHIP_LF_48"



Figure 27. Run 3 Incubation

6.2. Field Selection

Click on the 3rd tab, "Target Setup", and the 1st sub-tab, "Target". Target is the same for the whole workflow, but the field setup will need to be changed at every step.

Load the field "LF_48_Run_3&4_Incubation&Cooldown" which will spot 315 drops (~120 nL) per well. To load the field, click in "Target" Figure 17 >> "Load Field", select the field corresponding to the run (Figure 28) and you will have the field corresponding to the Figure 29.



5-	E Target Field Task List Help Open Target Selection Window Ia Load Field Save Field	Run Robot	5etup H [%]: 52	T [°C] 22		celler
	Ta Copy Field					
	Select Field	No. of Fields	Spot per Field	Spot Pitch [um]	Auto Center	
	Field Offset Sort by Field Position Ignore Nozale Offset Parallel Solotting Line Spotting Spot on the Fly	X 1 Y 1 I	X 12 v Y 4 v 5	X 4500 v Y 4500 v	Auto Center X OFF/ON Auto Center Y OFF/ON	
	Selected Field 1		41	0.1 0.5 1.0	1.5 2.0 2.5 3.0	
	ed 1					
ľ	Name	^		Date modified	Туре	
•	Name IF_48_Run_1_MasterMix IF_48_Run_2_Cells	^		Date modified 9/29/2022 5:23 PM 7/8/2022 3:30 PM	Type A FLD File FLD File	

Figure 28. Load field for incubation

in Nozzle Setup	Target Setup	Run	Robot Se	etup H [%]: 56	T [℃] 22	BT [°C] 17	DP [°C] 13	cellenONE mode
rget Field Setu	P							
1 Telect	ed Nozzle							
				Set 🤝	(All)			
					\sim		Field 1	
							1 2 3	4 5 6 7 8 9 10 11
fTP384			Position	Probe	Drops		000	000000000
			1/1	141	315		1 0 000	
			1/2	1A1.	315.			
			1/3	1A1.	315.		3 0 000	
		I	1/4	1A1,	315,		4 0 000	
	+++++	I	1/5	1A1,	315,		Jaco Jacobio	
			1/6	1A1,	315,			
			1/7	1A1,	315,			
			1/8	1A1,	315,			
			1/9	1A1,	315,			
			1/10	1A1,	315,			
			1/11	1A1,	315,			
			1/12	1A1,	315,			
			2/1	1A1,	315,			
			2/2	1A1,	315,			
		I	2/3	1A1,	315,			
			2/4	1A1,	315,			
			2/5	1A1,	315,			
			2/6	1A1,	315,			
			2/7	1A1,	315,			
			2/8	1A1,	315,			
			2/9	1A1,	315,			
			2/10	1A1,	315,			
A Well			2/11	1A1,	315,			
Plate No.	1		2/12	1A1,	315,			
riace no.			3/1	1A1,	315,			
No. of Drops	1		3/2	1A1,	315,			
			3/3	1A1,	315,			
Well Order	Pause Se	ttings	3/4	1A1,	315,			
					1 DATE			

Figure 29. Field setup for incubation

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6.3 Start the Run

Start the run and **select all 3 chips**, then click OK (Figure 30).

Warning. Please make sure that the 3 proteoCHIPs are selected regardless of the number of chips processed.

Hain Nozzle Setup Target Setup Run Robot Setup H [%]:47 T [%] 23 BT [%] 20	🗘 Select Run Directories — 🗆 🗙	<u>5</u>
Probe MITP384 Run	Directory for Run Folders	Stilleder 1 0.0 40.0 40.0 10000 1000 1000
2 StartRun LF_48_Run_3_Incubation_50C Stop Target proteo(HIP_LF_48 Nozzłe Setup	3 Incubation 50C	proteoCHIP 3
Field Name (usist loaded of salved) UF-84 Sun, 384 Jinubaton&Cooldown. fid Ignore Nozde Offset market Spotting Sort By X Field Position Start Time: Estimated Completion Time:	Volume limit Sample Volume [µi] 10 Save Images None Y Enrichment	proteoCHIP 2
Plate No. Well Nozzle Drops Target No. Field No. X Pos. Y Pos.	Auto Drops to Discard \$ 5	proteoCHIP 1
•	Show this dialog before each run (to enable, open from menu)	· ·

Figure 30.Start the run for Incubation

Once the Run has started, humidity and temperature are automatically set. A pop-up message will be displayed, click "OK" to start the 1h30 incubation (Figure 31).

➔ Dialogue	Comment ×	Comment ×
Please, insert MTP384 probe and press "OK". WARNING - The PDC is going to uptake. Please do not open the door. Risk of PDC breakage!	Please make sure that the proper target setup A is loaded - Run 3 - Then press CONTINUE	Incubation at 50 C will start for 1.5 hours. After 1.5 hours, when you are ready to proceed to Run 4, Click "Stop Run" and start Run 4
✓ OK Abort	CONTINUE	ОК

Figure 31. Incubation messages

After 1h30, once you are ready to proceed to the next step, click the red "Stop" button. Dismiss the pop-ups by clicking "Exit Run" and then "Partially spotted" (Figure 32).

	Probe MTP384	Dialogue		🗘 Dialog	
Start Run	Run Run_3_Incubation_1h_50C Stop Target proteoCHIP_LF_48 Nozzle Setup	Run is paused! Do you want to continue or abort?	^	Run was aborted. Choose the status of the current probe(s).	^
Start Time: 3:28 PM	Field Name (Bast loaded or saved) Run3_Incubation_3Chips_SOC_4Smin.ftd re Nozzie Offset Braild Spotting M- 10/5/2022 Estimated Completion Time: 1:00 AM - 1/1/1504 Nozzie Drops Target No. Field No. X Pos. Y Pos.		v		,
Comment		Continue Exit run		Partially spotted Not Spotte	d

Figure 32. Exit the run



7. Step 4: Cooldown

Following incubation at 50 °C, we need to bring the plate temperature back to 20 °C. During this process, the system will keep on rehydrating the wells continuously. The cooldown step takes approximately 30 minutes to complete.

This run needs to be launched **immediately** after stopping the previous run, as in the meantime rehydration cycles are interrupted, but the temperature setting is still 50 °C. Not starting the Cooldown run immediately can result in sample evaporation.

7.1. Probe, run and target selection

In the Main tab of the software, select the probe, run and target to be used (Figure 33).

- Probe: "MTP384"
- Run "LF_48_Run_4_Cooldown_20C"
- Target: "proteoCHIP_LF_48"

No changes need to be made in the field setup section, so you can proceed to start the run immediately.

💭 Main						
File Target Field Task List	Help					
Main Nozzle Setup T	arget Setup	Run Robot Setup	H [%]: ⁴⁵ T [%	C] 24 BT [°C	C] 21 DP [º	C] 12
Probe MTP384 cellenWASH MTP_96 cellenVIALS cellenWASH_TALL	Prin Run LF_48 LF_48 LF_48 LF_48 Run_11 Run_3; SpotRu SpotRu SpotRu cellenC	Mounted No ime 8_Run_1_MasterMix 8_Run_2_Cell_Isolation 8_Run_3_Incubation_50C 8_Run_4_Cooldown_20C 8_Run_5_Dilution_1chip 1_MasterMix_300nLTest 3p_Incubation_1h30_50C Run Run_Destatic nONE_Basic	zzles P P	arget ITP384_scienion roteoCHIP_LF_4% roteoCHIP_LF_4%	3 3_ManualI 3_Copy	
			Field	Name:		
Run Task		Run Con	nment			
Message_LF_Run4_Incuba Message_LF_Run4_CoolDo Humidity_85 BeginLoop Dip_into_Washstation1_Or SpotProbeRun	tion_20C_TargetS wm nce	tSetup			^	
EndLoop	- 20				~	

Figure 33. Run 4 Cooldown to 20 °C

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7.2. Start the run

Once the run has been selected, click "Start Run" in the Run tab. Select all 3 chips regardless of the number of loaded chips and click "OK".

The run will finish once the temperature of 20 °C is reached. This should take approximately 30 minutes.

Mair	Nozzle Setup	Target Setup Run Robot Setup H [%]: 47 T [°C] 23 BT [°C] 2	🛛 🔿 Select Run Directories — 🗆 🛛 🛛	<u>25 min</u>
		Probe 1	Directory for Run Folders	Section All Copy Heads Temper Example Image: Section All Copy Heads Temper Example Image: Section Temper Example Temper Example Temper Example Image: Section Temper Example Temper Example Temper Example Image: Temper Example Temper Example Temper Example Temper Example
2	Start Run	Target ProteoCHIP_LF_48 December 2000 Decemb	Batch Identification CoolDown	, proteoCHIP 3
	Ignor Start Time:	Field Hame (last backed or saved) UE-48, RUL 34/Linokatorik Cookdown.fd re Nozde Offset Parallel Sporting Estimated Completion Time:	Volume limit Sample Volume [µ] 10 Save Images None Enrichment Hultidrops	proteoCHIP 2
	Plate No. Well Comment	Nozzle Drops Target No. Field No. X Pos. Y Pos.	Auto Drops to Discard \$ 5	proteoCHIP 1
		•	Show this dialog before each run (to enable, open from menu)	

Figure 34. Starting the run for Cooldown

Comment	×	Comment ×
The system will now cool down to 20 C. Once this temperature is reached, you may start Run 5. This will take about 30 minutes.	^	Please make sure that the proper target setup A is loaded - Run 4 - Then press CONTINUE
	~	~
ОК		CONTINUE

Figure 35. Pop-up messages

Comment	~	Comment	
Incubation completed !	^	Before next run, make sure that you select cellenWASH as the source plate and that you load the correct target setup - Load 250uL of 0.1% TFA/FA in the cellenWASH1	^
	~		~
ОК		CONTINUE	

Figure 36.Incubation completed



8. Step 5: Dilution

The dilution step is used to adjust the end volume of the final sample that is going to be injected in the LC. The final volume should be around 3.5 μ L to be correctly injected into the HPLC system loop before the MS analysis. To achieve that, 0.1% FA or 0.1% TFA is added to each well of the chip.

This step can either be performed manually or by an automated cellenONE run.

8.1. Manual dilution

Please make sure that the oil is liquid (temperature should be above 18 °C). Pipette 3.2 μ L of 0.1% FA or TFA in each well of the chip. Optional: Use a multichannel pipette.



Figure 37. Manual dilution using a multichannel pipette

8.2 cellenONE automated dilution

8.2.1. Probe, Run and Target selection

In the Main tab of the software, select the probe, run and target to be used (Figure 38).

- Probe: "cellenWASH" / "cellenWASH_TALL"
- Run: "LF_48_Run_5_Dilution_1chip"
- Target: "proteoCHIP_LF_48"



🔿 Main

File Target	Field Task L	ist Help										
Main Noz	zle Setup	Target S	etup	Run	Robot Setup	H [%]: 48	T [ºC]	24	BT [℃]	20	DP [°C] 12
Probe MTP384 cellenWAS MTP_96 cellenVIAL cellenWAS	SH SH_TALL		Pr E.4 LF_4 LF_4 LF_4 LF_4 LF_4 Run_ Run_ Spott Spott celler	ime 8_Run_1 8_Run_2 8_Run_4 8_Run_4 8_Run_9 1_Maste 3p_Incu Run Run_Des nONE_Ba	Mounted I - MasterMix 2 Cell_Isolation 3 Incubation_50C + Cooldown_20ch 5 Dilution_1chip rrMix_300nLTest bation_1h30_50C ttatic usic	lozzles	Field Na	get 384_sc eoCHIF eoCHIF	ienion P_LF_48 P_LF_48_0 P_LF_48_0	ManualI Copy		
Run Task					Run Ce	omment						
Message_ Uptake_ce Intermedia BeginLoop SpotProbe Pump_3-2 EndLoop	LF_Run5_Dilu ellenWASH_1(ate_Pump_3-) eRun uL	ution_Target 60uL 2uL_before_	Setup 1Spot									

Figure 38. Run 5 Dilution

8.2.2. Field Selection

In the Target tab, load the field "LF_48_Run_5_Dilution" (Figure 39).

	Load Field Save Field Comunication	Run Robot	Setup H [%	6]: 52	T[°C] 22		cellen
s	Select Field Select Field Select Field Field Offset Sort by Field Position Ignore Nozale Offset Paralel Spotting Line Spotting Spot on the Fly Selected Field 1	No. of Fields X Y 1 i	Spot per X 12 Y 4	Field	Spot Pitch [µm] X 4500 • Y 4500 • • 0.1 0.5 1.0	Auto Center Auto Center X OFF/ON Auto Center Y OFF/ON 1.5 2.0 2.5 3	.0
Та	rget Image χ 0 γ 0						
	no 1		· ·				
	Name				Date mo	dified	Туре
3	Name I LF_48_Run_1_MasterN I LF_48_Run_2_Cells	 			Date mo 9/29/202 7/8/2022	dified 22 5:23 PM 2 3:30 PM	Type FLD File FLD File

Figure 39. Load field for Dilution



ain	Nozzle Setup	Target Setup	Run	Robot Sel	tup H [%]: 56	T [℃] 23	BT [°C] 11	DP [°C] 14	cellenONE mode
Targe	• Field Setup	7							
	1			_					
_1	Selected	i Nozzle			Col.				
					Set			Field 1	
								TICIU I	
celler	nWASH			-		1.5		123	4 5 6 7 8 9 10 11 1
	-			Position	Probe	Drops			000000000
				1/1	1A1,	1,	_		0000000000
		CI I		1/2	101	1		1 1 8 8 9 9	000000000000000000000000000000000000000
	— —			1/4	141,	1		4 H 866	000000000000000000000000000000000000000
				1/5	141.	1.		. 12 1000	
L .				1/6	101,	1.			
				1/7	1A1.	1.			
L .				1/8	1A1.	1.			
L .				1/9	1A1.	1.			
				1/10	1A1,	1,			
				1/11	1A1,	1,			
L .				1/12	1A1,	1,			
				2/1	1A1,	1,			
				2/2	1A1,	1,			
				2/3	1A1,	1,			
				2/4	1A1,	1,			
				2/5	1A1,	1,			
L .				2/6	1A1,	1,			
				2/7	1A1,	1,			
				2/8	1A1,	1,			
				2/9	1A1,	1,			
				2/10	1A1,	1,			
Α	Well			2/11	1A1,	1,			
	Plate No.	1	-	2/12	1A1,	1,			
			-	3/1	1A1,	1,			
No	of Drops	1		3/2	1A1,	1,			
	w.to.t.			3/3	141,	1,			
	Weil Order	Pause Set	tungs	2/5	1041,				

Figure 40. Target setup Dilution

8.2.3 Start the run

Once you chose the right Probe, Run, Target, and you loaded the dedicated field, click "Start Run" in the Run tab (Figure 41). A pop-up window will prompt you to insert a cellenVIAL or a pre-taught PCR tube (cellenWASH_TALL) loaded with 250 μ L of 0.1% TFA or FA in the cellenWASH1 position (Figure 41). Once this is done, click "OK" to continue. If the cellenWASH_TALL is not taught on your machine and you would like to use a PCR tube, please contact your Field Application Specialist.

Main Nozzle Setup Target Setup Run Robot Setup H [%]: 45 T [*C] 23 BT [*C] 20 O Select R	n Directories - O X
Probe CollenWASH	ing for Run Folders Ingentiate In
Start Run	Identification jon proteoCHIP 3 Imme limit _ Sample: Volume [pl] 10 0
Ignore Nozie Office Parallel Spotting Sort By X Field Position Save Ima Start Time: Estimated Completion Time:	pes None Enrichment ProteoCHIP 2
Plate No. Viel Nozzle Drops Target No. Field No. X Pos. Y Pos.	Auto Drops to Discard \$ 5 Cancel his dialog before each run (to enable, open from menu)



Figure 41. Starting the Dilution run



9. Sample transfer

Place the holder containing the chip(s) on ice so the temperature decreases, and the oil becomes solid (temperature below 18 °C) (Figure 42).

Once the oil is solid (after ~5 minutes), it is easy to pipette out 3.5 μ L of the sample droplet. The solidified oil will keep sticking onto the chip and will not be transferred with the sample to be analysed.

You can now transfer your samples to the desired injection plate/vials, etc.



Figure 42. Solid oil in proteoCHIP LF 48

Note. For the moment this step is performed manually. We are currently working on implementing direct injection from the chip to most of the common autosamplers available. As soon as this feature is available, we will let you know!