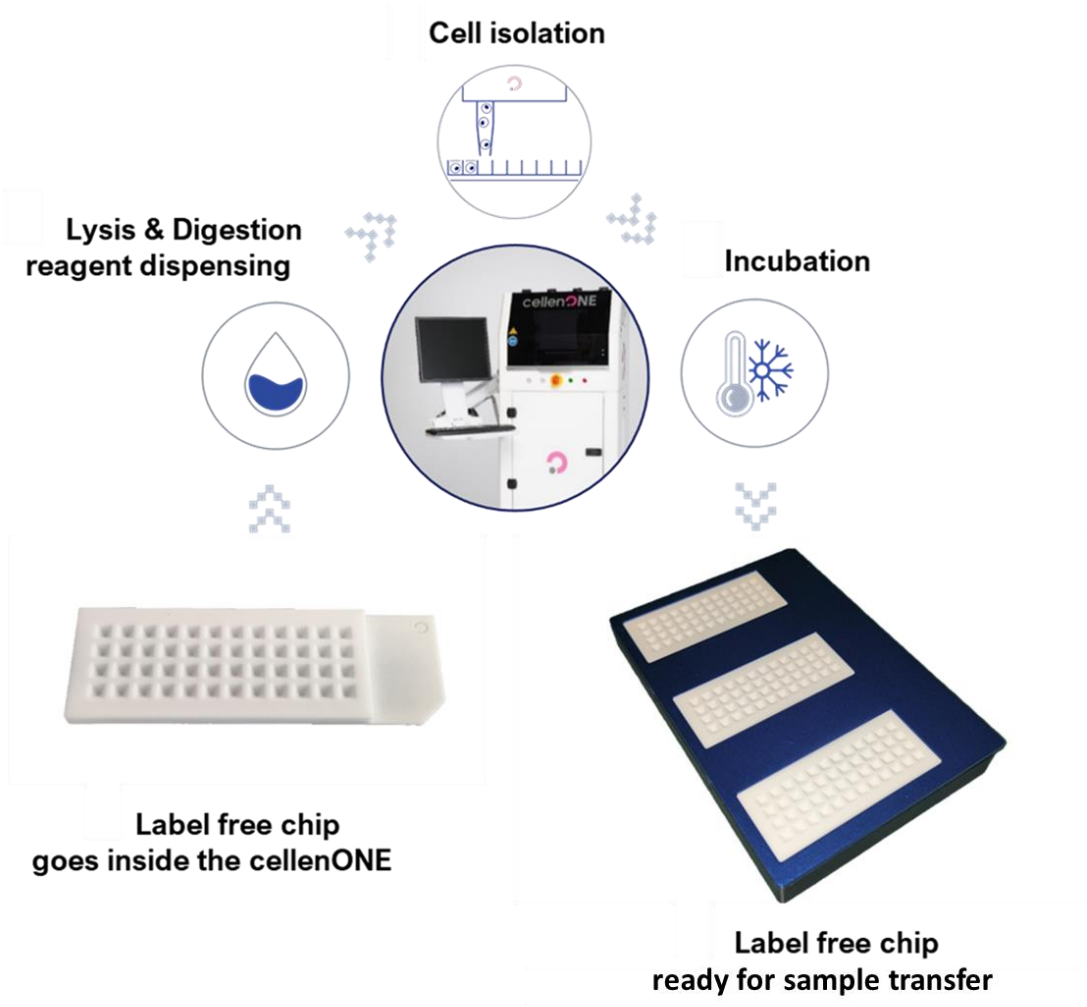
Quick Start Guide
proteoCHIP LF 48

for Label Free
Single Cell Proteomics workflow
using the cellenONE®



General remarks:

- Wipe the inside of the enclosure and door with isopropanol or 70% ethanol as well as the proteoCHIP LF 48 holder.
- When manipulating inside the cellenONE, make sure that you are wearing gloves and a lab coat. No skin should be exposed inside the enclosure to avoid contamination.
- Avoid opening the door as much as possible during the experiment.
- Make sure the PDC is aligned to the red cross, and you have a stable drop.
- Measure the drop volume.
- Set the temperature to 10 °C.
- Place the proteoCHIP(s) inside the cellenONE holder. If processing only one chip, insert it on the spot closest to you.
- Place the 'proteoCHIP holder top' on top of the 'holder' and insert the assembled unit on the target holder inside the instrument.
- All solutions should be prepared in a biosafety cabinet.

Note. Please make sure that the 14 days wash have been performed previously (every 14 days), clean the humidifier with 70% ethanol every 14 days, if possible, clean the PDC with sciClean at the end or beginning of every experiment followed by 3 flushes (Flush_PDC).

Run 1 – Master mix dispensing:

- Prepare 100 µL of Master mix: 10 µL 1M TEAB + 60 µL H_2O + 20 µL 1% DDM + 10 µL 100 ng/µL Enzyme(s) and load at least 60 µL into a well of a 384-well plate. If you decide to do the optional drop optimization step, load 70 µL.
- Check if the drop is stable.
- Optional: Aspirate 10 µL of Master mix and optimize the Voltage and Pulse to have a stable drop. You may need a higher Voltage (+5-10%).
- Measure the drop volume.
- Probe: "MTP384"
- Run: "LF_48_Run_1_MasterMix"
- Target: "proteoCHIP_LF_48"
- Load the field "LF_48_Run_1_MasterMix".
- Update the Field table: correct probe well and correct volume: 300 nL.
- Check if the drop is stable.
- Start the run.

Run 2 – Cell isolation:

- Probe: “MTP384”
- Run: “LF_48_Run_2_Cell_Isolation”
- Target: “proteoCHIP_LF_48”
- Load the field “LF_48_Run_2_Cells”.
- Update the Field table: correct probe well and adjust the number of cells if needed.
- Load ~ 30 µL cell sample into a well of a 384-well plate.
- Check if the drop is stable.
- Start the run.
- When the run is automatically paused after probe uptake:
  - check if the drop is stable,
  - check the detection parameters,
  - perform a mapping,
  - adjust the isolation parameters.
  - Continue the run.
- Flush the PDC once the isolation is complete.

Run 3 – Incubation at 50 °C (minimum 1 h 30 min):

- Probe: “MTP384”
- Run “LF_48_Run_3_Incubation_50C”
- Target: “proteoCHIP_LF_48”
- Load the field “LF_48_Run_3&4_Incubation&Cooldown”.
- Check if the drop is stable.
- Measure drop volume.
- Update the Field table: correct probe well and correct volume: 120 nL.
- Start the run.
- Select all 3 proteoCHIPs.
- After 1 h 30 min, when ready, stop the run and proceed **immediately** to Run 4.

Run 4 – Cooldown to 20 °C (~ 30 min):

- Probe: “MTP384”
- Run: “LF_48_Run_4_Cooldown_20C”
- Target: “proteoCHIP_LF_48”
- Start the run.
- Select all 3 proteoCHIPs.

Run 5 – Dilution:

- Manual:
  - Make sure the oil is liquid.
  - Pipette 3.2 µL of 0.1% FA or TFA in each well of the chip.
- cellenONE:
  - Probe: “cellenWASH” / “cellenWASH_TALL”
  - Run: “LF_48_Run_5_Dilution_1chip”
  - Target: “proteoCHIP_LF_48”

  - Load the field “LF_48_Run_5_Dilution”.
  - Load 250 µL 1% TFA or FA into a cellenVIAL or appropriate PCR tube if a cellenWASH_TALL probe is available on your machine.
  - Start the run and repeat it for each proteoCHIP.

Sample transfer:

- Place the holder containing the chip(s) on ice so the oil solidifies.
- Pipette out 3.5 µL of the sample droplet.