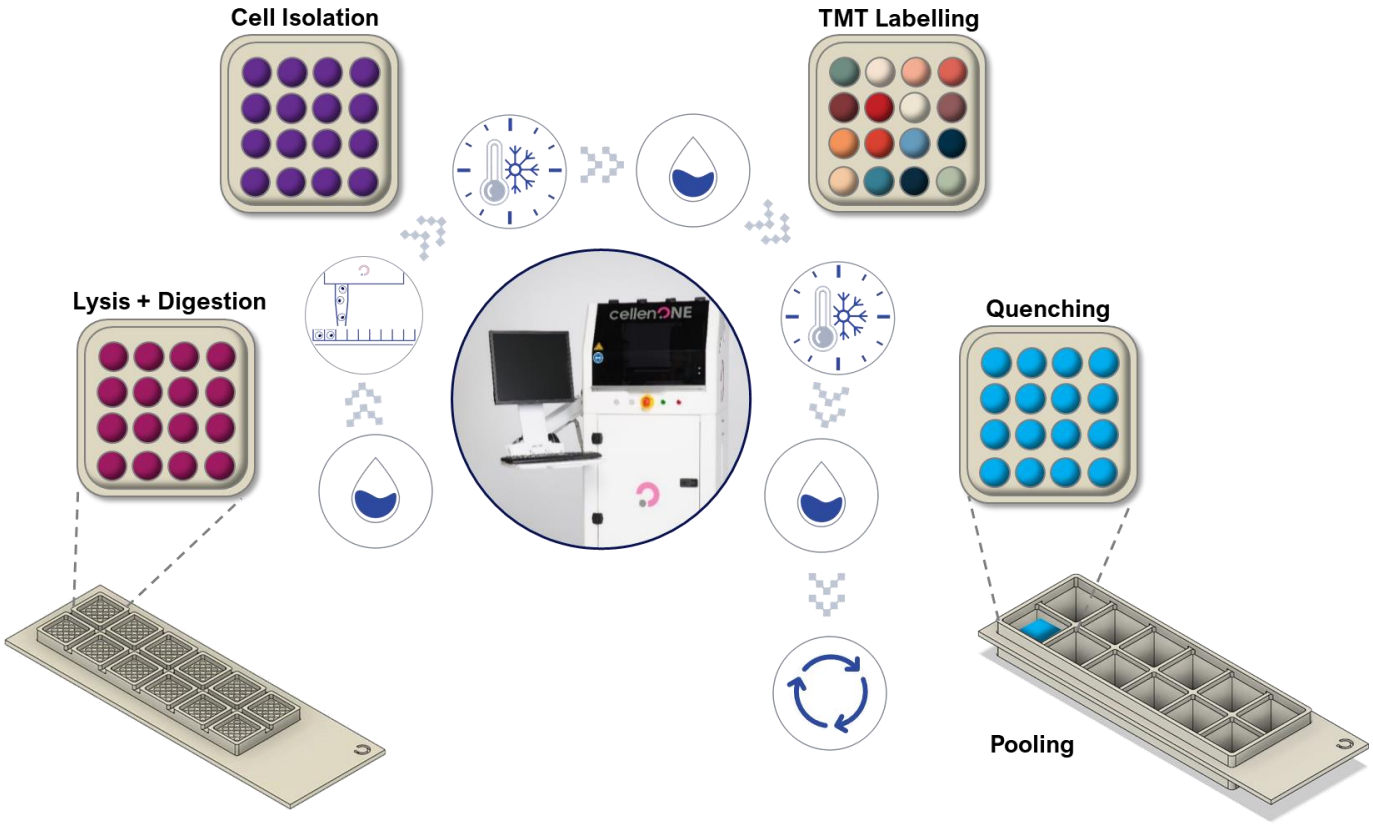
Quick Start Guide
proteoCHIP 12*16

for multiplexed
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 12*16 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 produces 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 50 µL into a well of a 384-well plate. If you decide to do the optional drop optimization step, load 60 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: "12x16_Run_1_MasterMix"
- Target: "proteoCHIP_1216"
- Load the field "12x16_Run_1_MasterMix".
- Update the Field table: correct probe well and correct volume: 50 nL.
- Check if the drop is stable.
- Start the run.

Run 2 – Cell isolation:

- Probe: “MTP384”
- Run: “12x16_Run_2_Cells”
- Target: “proteoCHIP_1216”
- Load the field “12x16_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.

Run 3 – Master mix dispensing:

- Load at least 50 µL Master mix into a well of a 384-well plate. If you decide to repeat the optional drop optimization step, load 60 µL, otherwise use the Voltage and Pulse values you obtained before the first Master mix dispensing.
- 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: “12x16_Run_3_MasterMix”
- Target: “proteoCHIP_1216”
- Load the field “12x16_Run_3_MasterMix”.
- Update the Field table: correct probe well and correct volume: 50 nL.
- Check if the drop is stable.
- Start the run.

Run 4 – Incubation at 50 °C (minimum 2 h):

- Probe: “MTP384”
- Run “12x16_Run_4_Incubation_50C”
- Target: “proteoCHIP_1216”
- Load the field “12x16_Run_4&5_Incubation&Cooldown”.
- Check if the drop is stable.
- Measure drop volume.
- Update the Field table: correct probe well and correct volume: 30 nL.
- Start the run.
- Select all 3 proteoCHIPs.
- After 2 hours, when ready, stop the run and proceed to Run 5.

Run 5 – Cooldown to 20 °C (~ 30 min):

- Probe: “MTP384”
- Run: “12x16_Run_5_Cooldown_20C”
- Target: “proteoCHIP_1216”
- Check if the drop is stable.
- Start the run.
- Select all 3 proteoCHIPs.
- Make sure the temperature is at 20 °C.

Run 6 – TMT labelling:

- Thaw TMT labels (aliquoted and stored at -80 °C, 5 µg/µL).
- Make a note of the Voltage and Pulse.
- Optimise the drop for acetonitrile with the AirGapTakeProbe_40µl task and Continuous Dispensing. The general starting point should be decreasing the Voltage by 10 and increasing the Pulse by 5.
- Flush the PDC.
- Check if the drop is stable.
- Probe: “MTP384”
- Run: “12x16_Run_6_TMT_1or2chips” or “12x16_Run_6_TMT_3chips”
- Target: “proteoCHIP_1216”
- Load the field “12x16_Run_6_Labelling_Nplex”.
- Update the Field table: correct column of probe wells.
- Start the run.

- During the run you will be prompted by messages to load sequentially 55 µL of pure acetonitrile into the selected wells and 15 µL of TMT label into the wells on the right.
- At the end of this run, an incubation of 30 minutes at RT will start.

Run 7 – Quenching:

- Prepare 100 µL of quenching solution: 96 µL H_2O + 3 µL 37% HCl + 1 µL 50% Hydroxylamine and load 50 µL into a well of a 384-well plate.
- Put the Voltage and Pulse values back as before the TMT run.
- Probe: “MTP384”
- Run: “12x16_Run_7_Quenching”
- Target: “proteoCHIP_1216”
- Load the field “12x16_Run_7_Quenching”.
- Measure drop volume.
- Update the Field table: correct probe well and correct volume: 50 nL.
- Start the run.
- At the end of this run, an incubation of 15 minutes at RT will start.

Run 8 – Dilution:

- Manual:
  - Pipette 2.5 µL of 0.1% TFA/FA into each funnel and proceed to the next step: Pooling.
- cellenONE:
  - Probe: “MTP384”
  - Run: “12x16_Run_8_Dilution_1chip”
  - Target: “proteoCHIP_1216”
  - Load the field “12x16_Run_8_Dilution”.
  - Update the Field table: correct probe well and correct volume: 150 nL (in every well regardless of the number of TMT labels used).
  - Load 50 µL of 0.1% TFA/FA into a well of a 384-well plate.
  - Check if the drop is stable.
  - Start the run and repeat it for each proteoCHIP.

Pooling:

- Place the proteoCHIP Funnels on top of the proteoCHIPs and take the assembled unit out of the cellenONE
- Transfer the proteoCHIPs + Funnels into the centrifuge adaptor with the Funnels at the bottom of the centrifuge holder and the proteoCHIP on top. Then close the lid of the centrifuge holder.
- Use a plate centrifuge to pool the arrays into the funnel at 1500 rpm for 2 minutes at room temperature.
- Take out the proteoCHIP with the funnel from the centrifuge adaptor and keep the funnel without the proteoCHIP.
- After pooling, the 8- to 16-plex samples are in each funnel and can be directly injected into the LC from the funnels (refer to the Autosampler and direct injection manual for instructions). In that case, seal the funnels with aluminium foil and store them at 4 °C (can be done directly inside the autosampler) for at least 10 minutes before starting injection (to allow the oil layer to become solid).

Funnel layout after pooling

Sample transfer:

- Manual:
  - Place the funnels on ice so the oil solidifies.
  - Pipette out 5 µL of the sample droplet.
- Autosampler:
  - Direct injection is possible using the Thermo Scientific UltiMate 3000 UHPLC, with which the injection height is easily adjustable. To set your autosampler for direct injection from the proteoCHIP funnels please refer to the Autosampler User Manual.