# Sample lysis & preparation for SP3

IMPORTANT:

- The SP3 sample preparation is only available for specific projects and only upon agreement with the staff of the Proteomics Core Facility.
- fill out the submission form online when you hand over the samples (ask for help in the facility)

## Reagents & Solutions

- SP3 lysis buffer: 5% SDS in 1x PBS *
- 2x SP3 lysis buffer: 10% SDS in 1x PBS *
- Dithiothreitol (DTT), 100 mM stock *
- Chloroacetamide (CAA), 550 mM stock *
- Benzonase HC nuclease *

## Equipment

- Benchtop Eppendorf centrifuge *
- Eppendorf thermo mixer *
- Diagenode Bioruptor
- DirectDetect protein concentration kit *

* available in the core facility

## Protocol

**Please work under the clean bench, wear gloves & work as clean as possible!**

1. Sample lysis[1]
    a) CELL PELLETS. Lyse PBS-washed cell pellet in SP3 lysis buffer. Rule of thumb: the volume of the lysis buffer should equal the volume of your cell pellet. Lyse cells by pipetting. Continue with step 2
    b) TISSUE SAMPLES. Lyse tissue in SP3 lysis buffer. Rule of thumb: the volume of the lysis buffer should equal the volume of your tissue sample. Mix carefully by pipetting. Heat for 10 min at 95°C. Continue with step 2
    c) NON-DENATURED PROTEIN SAMPLES. If your purified proteins or organelles (e.g. mitochondria) are in a non-denaturing buffer (e.g. Sucrose/Mannitol) add the same volume of 2x SP3 lysis buffer to the sample. Mix by pipetting. Heat for 5 min at 95°C. Continue with step 3 (in case of enriched nuclear fraction continue with step 2)
    d) DENATURED PROTEIN/Co-IP/PULLDOWN. If you have your protein already in a denaturing buffer (containing either SDS, Urea, SDC etc., for instance Laemmli buffer) or in case you have eluted your protein from any type of bead continue with step 3
    e) FACS SORTED CELLS. Add an equal volume of 2x SP3 lysis buffer to your cell suspension. Lyse by pipetting. Continue with step 2
    f) LASER CAPTURE MICRODISSECTED SAMPLE. Collect the microdissected cells or tissue chunks directly into the SP3 lysis buffer. Heat for 10 min at 95°C.[2] Continue with step 2

2. Degrade chromatin using a Bioruptor (10 min, cycle 30/30 sec) or equivalent sonifier. ALTERNATIVE: Completely degrade all nucleic acids by Benzonase HC. Use **25 Units per 500.000** cells.[3] Mix briefly and incubate for 30 min at 37°C. Check the viscosity of the sample to ensure that the chromatin has been completely degraded

3. Add DTT to a final concentration of 5 mM, vortex and incubate at 55°C for 30 min

4. Add CAA to a final concentration of 40 mM, vortex and incubate in the dark for 30 min at room temperature

5. Centrifuge for 10 min at 20.000 × g. Transfer the supernatant to a new tube in case a pellet is visible

6. Measure protein concentration by your method of choice (or the DirectDetect kit), it should ideally be ≥ 1 µg/µL.[4] The maximum sample volume at this point is 100 µL. Contact the facility staff if your sample volume is larger

7. Freeze your samples at -20°C. Hand them over to the Proteomics Core Facility and fill out the online sample submission form

## Notes

[1] Check the starting volume of your samples and calculate how much volume you need to add at each step. Final volume should not exceed 100 µL.

[2] Increase the incubation time at 95°C to 30 min if your tissue has been preserved with (para)formaldehyde or any other crosslinking agent.

[3] If the amount of cells/input sample is unknown dilute 1 µL Benzonase HC with 9 µL MilliQ water. Add 1 µL of this dilution to your sample. Store the diluted benzonase at 4°C but no longer than 24h.

[4] Do not measure protein concentration if you only have minute amounts of sample, i.e. if the protein concentration measurement would consume already large parts of your total sample.