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Preparation of cell free extracts for enzyme determinations

Freeze buffer (1 liter)
- Make 1 liter of 10 mM K_2HPO_4
- Make 500 ml of 10 mM KH_2PO_4
- Take about 800 ml of 10 mM K_2PO_4
- Adjust pH to 7.5 by adding 10 mM KH_2PO_4
- Add 0.75 g EDTA to 1 liter

Sonication buffer (1 liter)
- Make 1 liter of 100 mM K_2HPO_4
- Make 500 ml of 100 mM KH_2PO_4
- Take about 800 ml of 100 mM K_2PO_4
- Adjust pH to 7.5 by adding 100 mM KH_2PO_4
- Add 0.4 g $MgCl_2$ (2 mM) to 1 liter

Sampling
- Collect the equivalent of 30-40 mg biomass dry weight of cell culture in a 50 ml falcon tube
- Centrifuge 2 minutes at maximum speed at 4°C
- Discard supernatant
- Wash cells twice in freeze buffer (see below)
- Centrifuge 2 minutes at maximum speed at 4°C
- Resuspend pellet in 2 ml 'freeze buffer' and split the sample in two samples of 1ml
- Store in -20°C freezer

Preparation extracts
- Thaw samples
- Centrifuge for 2 minutes at maximum speed at 4°C
- Wash the pellet with 1 ml of ice-cold sonication buffer
- Centrifuge again and resuspend the cells in 1 ml of ice-cold sonication buffer and add 10 µl 0.1 M DTT
- Transfer sample to a pre-cooled screw-lock tube with 0.75 g glass beads (425-600 microns)
- Shake samples 8 bursts of 10 sec at speed 6 in the Fast prep machine
- Cool samples inbetween bursts for at least 60 sec on ice
- Centrifuge 10 minutes at maximum speed at 4°C
- Transfer supernatant to fresh tube (this is the cell free extract)
- Dilute samples in sonication buffer with the same concentration of DTT:

Dilution	Cell free extract in µl	Sonication buffer + DTT in µl
undiluted	200	0
2x	100	100
4x	50	150
8x	25	175

- Keep samples on ice

Enzyme assays

- see separate protocols

Total protein content determination – BCA protein assay

- Add 2 µl of 0.1 M DTT to 200 µl BSA stock (of 2 mg/ml)
- Make the following standard solutions for a calibration curve:

# Standard	BSA solution + DTT in µl	Sonication buffer + DTT in µl	BSA conc. (µg/ml)
blanc	0	40	0
1	5	35	250
2	10	30	500
3	15	25	750
4	20	20	1000
5	25	15	1250
6	30	10	1500
7	40	0	2000

- Pipette 10 µl of each standard, diluted and undiluted samples (use the previously made dilutions) into a 96-wells plate (flat bottom)
- Make the BCA reagent according to the instructions of the manufacturer
- Add 200 µl BCA reagent to every well with standard or sample
- Cover plate with parafilm
- Incubate for 30 minutes at 37°C
- Determine absorbance at wavelength 570 nm with the Novostar