LIMS_SOPs_measurements_metabolites_quenching_ionchromatography_2.txt

= Writing SOPs =
[[PageOutline]]

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== Abstract ==

This document describes how quenching of the metabolism and the extraction of intracellular metabolites can be performed.

The quenching method was established according to:

  * Link et al. (Leakage of adenylates during cold methanol/glycerol quenching of 'Escherichia coli', Metabolomics 2008 4:240-247)

The extraction and detection method were established according to:

  * J.B. Ritter et al. (Simultaneous extraction of several metabolites of energy ... , Anal Biochem. 2008 Feb 15;373(2):349-69)

== Content ==

== quenching procedure ==

Quenching is a theme that is intensively discussed under metabolome researchers. The method present here was advised by Link et al. (2008) and seems to retain the cell integrity. It was conclude that quenching with 60%/40% methanol-glycerol is a good method when it comes to analysis of intracellular metabolites in Escherichia coli. One should always keep in mind that leakage is a big problem that might not be solved.

1. prepare a methanol-glycerol (100% glycerol stock solution) buffer (60%/40% v/v), propound 20 ml in a syringe and freeze the syringe and the buffer at -20°C [[BR]]
2. take 10 ml sample of an continuous culture, mix the sample carefully and keep it on dry ice until it is cooled to about -50°C [[BR]]
3. centrifuge the sample for 30 minutes, -19°C and at 10000x g [[BR]]
4. remove the supernatant and keep it for determination of the degree of leakage [[BR]]
5. wash the cell pellet with 10 ml of methanol-glycerol buffer (60%/40% v/v) and centrifuge again [[BR]]
6. remove the supernatant carefully, combine it with the first supernatant and keep the supernatant and the cell pellet in the fridge at -80°C [[BR]]

The cells should be extracted as soon as possible.

== extraction procedure ==

1. resuspend the cell pellet with 1 ml of methanol then quickly add 500 µl of chloroform and vortex vigorously, [keep on dry ice] [[BR]]
2. divide each sample by pipetting equal volume into 3 Eppis with 450µl propound chloroform and vortex all samples [[BR]]
3. add 900 µl of methanol/tricine (1mM) buffer (in 9:10 ratio) to each sample, vortex [[BR]]
4. centrifuge the sample for 10 min with 16000x g at 4°C [[BR]]
5. remove 800 µl of the upper (hydrophilic) phase and collect it in a separate Eppi [[BR]]

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6. add 800 µl of methanol/tricine (1mM) buffer (in 9:10 ratio) to the rest of the sample

7. centrifuge the sample again for 10 min with 16000x g at 4°C

8. remove the whole supernatant and combine it with the first supernatant [BR]

9. boil the sample for 4 min at 90°C to remove soluble proteins [use an Eppi-cap holder, if not the sample will be dispersed in your lab] [BR]

10. centrifuge the sample again for 10 min with 16000x g at 4°C and collect the supernatant [take care that you don’t transfer the protein pellet, too] [BR]

== concentration/pooling of samples ==

Due to the little concentrations of metabolites in the cells the samples have to be concentrated/pooled.

1. vaporize the methanol and water of the sample by aerating it with air or nitrogen until only a bit of glycerol is left [BR]

2. dissolve the metabolites with deionized water and filter the sample through a 0.2 µm filter

== Acceptable Variations ==

...

== Parts List ==

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== Changes to Previous Versions ==

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== Discussion and Comments ==