Microcystinase (MlrA) sample preparation for enzymatic activity assay

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Abstract

Samples for LC-MS analysis.

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Protocol

Step 1.

Add desired amount of microcystin (MC) to MlrA sample

- Look for appropriate amounts from articles (1 ug/ml MC used in a few at least)

- They will use 50 ul of the sample for the assay

- Negative control without enzyme is also needed for Viikki assay

- IMPORTANT!!!! USE GLASS TUBES AND PIPETS AT EVERY STAGE, MC has high affinity to plastic so even the storage of the sample has to be in glass tubes or vials. The enzyme might have some affinity to the glass, so use 0,1% BSA which will cover the glass walls so the enzyme won't have affinity to them.

Step 2.

Incubate 24 h in +37 °C

- If needed, take samples at different time points to verify that the peak of MC goes smaller

- Start in the morning

Step 3.

Samples can be stored in -20 °C

- If you freeze the samples then there is no need for inactivation

- If you don't freeze then add MeOH equal volume to the sample, these samples can be stored in RT or +4 °C, but keep them away from light