

ABRAXIS® Microcystins in Soil Sample Extraction

1. Intended Use

For the extraction of Microcystins from soil.

2. Sensitivity

0.15 ppb to 5 ppb using ABRAXIS® Microcystins ELISA Test Kits PN 520011, 520011OH or 522015
0.05 ppb to 5 ppb using ABRAXIS® Microcystins SAES ELISA Test Kit PN 520011SAES

3. Materials Required

15 mL glass centrifuge tubes and/or 20 mL glass vials
Water bath sonicator (Branson M1800 or equivalent)
Centrifuge capable of 1,500 x g
Nitrogen evaporator with heating module
Nitrogen
Strata X 200 mg columns with 6 mL volume (Phenomenex PNs 8B-S100-FCH or 8B-S100-FCL)
0.45 µm PVDF filters (Environmental Express PN SF145V)
5 mL Syringes (Becton Dickinson PN 309646 or equivalent)
Oven or incubator
Single channel 1-5 mL pipette with disposable tips or glass serological pipettes with pipettor
Analytical balance
ABRAXIS® Microcystins - ADDA ELISA Test Kit (PN 520011)
ABRAXIS® Microcystins - ADDA OH ELISA Test Kit (PN 520011OH)
ABRAXIS® Microcystins - ADDA SAES ELISA Test Kit (PN 520011SAES)
ABRAXIS® Microcystins - DM ELISA Test Kit (PN 522015)

4. Reagents Required (example volumes; can be adjusted to volume required using appropriate ratios)

- 4.1** Acidified 75% Methanol – Mix 750 mL Methanol, 250 mL Deionized water, and 6 mL 0.1 M Glacial acetic acid
- 4.2** 5% Methanol – 50 mL Methanol in 950 mL Deionized water
- 4.3** 90% Acetonitrile – 100 mL Deionized water in 900 mL Acetonitrile

5. Notes and Precautions

Before dispensing any volume of liquid, condition each pipette tip by drawing the liquid in and out of the tip 3 times before the final dispense. This will ensure that an accurate volume is transferred. Microcystins are susceptible to adsorption to plastic. For this reason, do not allow samples to remain in contact with any plastic material for an extended period.

6. Procedure

- 6.1** Weigh 1.00 g of soil sample into a 15 mL glass centrifuge tube or 20 mL glass vial.
- 6.2** Dry the sample by placing the open tube into an oven or incubator.
- 6.3** Add 5 mL of acidified 75% methanol.
- 6.4** Cap vial and place sample in a water bath sonicator for 25 minutes.

6.5 Centrifuge sample for 5 minutes at 1,500 x g.

6.6 Transfer supernatant to a clean, appropriately labeled glass tube or vial.

Note: If the glass vial or tube is not graduated, pre-mark at 1.5 mL (necessary for Step 6.8).

6.7 Add an additional 1 mL of acidified 75% methanol to soil sample. Repeat Step 6.5.

6.8 Transfer the supernatant and combine with the supernatant from step 6.6. Evaporate the combined supernatants to approximately 1.5 mL under a gentle stream of nitrogen at 60°C.

6.9 Dilute with 4.5 mL of deionized water.

Warning: If the diluted supernatant contains sediment, filter using 0.45 µm PVDF syringe filter to prevent the Strata X column from clogging in Step 6.11.

6.10 Condition Strata X column with 6 mL of methanol followed by 6 mL of water. Discard the methanol and water.

6.11 Pass entire sample (approximately 6 mL) through column. Discard the flow-through.

6.12 Rinse column with 2 mL of 5% methanol. Discard the rinse.

6.13 Elute sample using 5 mL of 90% acetonitrile. Collect in a clean, appropriately labeled glass vial.

6.14 Evaporate to dryness under a gentle stream of nitrogen.

6.15 Reconstitute sample with 1 mL of deionized water.

6.16 Filter sample with 0.45 µm PVDF syringe filters into a clean, appropriately labeled glass vial.

6.17 Analyze the sample extract according to Section G., *Assay Procedure*, of the selected ABRAXIS® Microcystins Plate ELISA kit user's guide.

7. Evaluation of Results

See Section H., *Evaluation*, of the selected ABRAXIS® Microcystins Plate ELISA kit user's guide. The results for the sample extract do not need to be multiplied by a dilution factor. If sample results exceed calibration, sample extract should be diluted further and re-analyzed to obtain accurate quantitative results.

8. Performance Data

Soil samples were spiked with various amounts of Microcystins, extracted as described above, and analyzed with the ABRAXIS® Microcystins-ADDA (PN 520011), ABRAXIS® Microcystins-ADDA OH (PN 520011OH), Microcystins ABRAXIS® (ADDA)-DM (PN 522015), and ABRAXIS® Microcystins-ADDA SAES (PN 520011SAES) kits. Average recovery was 92%.

9. Assistance

For ordering or technical assistance contact:

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