# Microcystins DM in Bivalves
# Extraction Protocol

## 1. Intended use

For the extraction of Microcystins from bivalve tissues using dilute methanol.

*Note: Microcystins may bind to plastics; efforts should be made to minimize extract contact with any plastic materials.*

## 2. Range of Detection

The range of detection of Microcystins in bivalve tissues is 3.75 ng/g to125 ng/g.

## 3. Materials required

Pipette(s) capable of delivering 1 mL - 5 mL and 20 µL - 200 µL
Methanol
Distilled or de-ionized water
Water bath
Thermometer
10 mL disposable syringes
0.45 µm syringe filters, PES membrane with pre-filter (Recommended: Environmental Express #SF145E)
Centrifuge capable of 3000rpm
20 mL or 40 mL glass vials with Teflon lined caps (must fit in centrifuge adapter)
10 mL glass graduated cylinders (one per sample)
Vortex mixer
Blender
ABRAXIS® Microcystin DM ELISA Kit (PN 522015)

## 4. Notes and Precautions

Shellfish must be shucked and uncooked.

## 5. Procedure

5.1 Heat water bath to 60°C, monitor and adjust as necessary throughout procedure to maintain temperature
5.2 Prepare sufficient quantity of 60% methanol; approximately 5mL needed per sample
5.3 Homogenize each shellfish sample thoroughly, cleaning blender well between samples
5.4 Weigh 1g of sample into glass vial
5.5 Add 2mL of 60% methanol to vial, vortex thoroughly
5.6 Place tube in water bath for 30 minutes
5.7 Centrifuge 10 minutes at 3000rpm
5.8 Decant supernatant into clean, labelled 10mL glass graduated cylinder and retain
5.9 Repeat steps 5.5 to 5.7 once, pooling the two supernatants in the graduated cylinder
5.10 Use 60% methanol to bring extract volume to 5mL, vortex
5.11 Transfer extracts to glass vials for storage using syringes and 0.45 µm syringe filters to remove any tissue particles
5.12 Before use in ELISA, combine 200 µl sample extract with 800 µl sample diluent
5.13 Sample is ready for analysis. Proceed to Section F of the ABRAXIS® Microcystin DM ELISA Kit User's Guide.

## 6. Evaluation of Results

The ELISA results must be multiplied by a factor of 25 to account for extraction dilution. Samples resulting in values lower than Standard 1 (0.15ppb) should be reported as <3.75 ng/g; results larger than Standard 5 (5ppb)

should be reported as >125 ng/g. Alternatively, samples resulting in values greater than Standard 5 may be further diluted to achieve quantitative results.

**7. For ordering or technical assistance contact**

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