

Imidacloprid: Plant Leaves Extraction Protocol

1. Intended use

For the extraction of Imidacloprid from plant leaf samples.

2. Limit of Detection

The limit of detection of Imidacloprid in plant leaf samples is 15 ng/mL (ppb).

3. Materials required

Scale or balance with at least 2-decimal capability

Disposable spatula

Glass centrifuge vials with screw caps to withstand 3000 x g centrifugal force

Pipette(s) capable of delivering 1 mL-5 mL, 20 µL-200 µL, and 10-15 mL with disposable plastic tips

5 mL and 10 mL serological pipettes

Methanol

0.45 µm pre-PES/Glass fiber syringe filters (optional)

Vortex mixer

Overhead tube rotator Centrifuge capable of 3000 x g

ABRRAXIS® Imidacloprid ELISA Kit (PN 500800)

ABRRAXIS® Imidacloprid Sample Diluent (PN 500801)

4. Notes and Precautions

- 4.1 Pipettes should be calibrated prior to performing extraction.
- 4.2 Prepare 50% methanol for extraction (i.e. 100 mL deionized water + 100 mL methanol).
- 4.3 All sample extractions and dilutions must be performed in glass vials.
- 4.4 To prepare sample, break up leaf sample and mix sample thoroughly to uniformity with a mixer, mortar and pestle or by hand with a spatula.

5. Extraction Procedure

- 5.1 Weigh 1 (+/-) 0.05 gram of sample into a 10-20 mL glass vial.
- 5.2 Add 10 mL of 50% methanol to sample (10-fold dilution). Screw cap on tightly and vortex or mix for 15 seconds.
- 5.3 Place tube on a rotator at room temperature for 10 minutes.
- 5.4 Use filter syringe with 0.45 µm filters or centrifuge tube for 5 minutes at 2500 x g (To prevent the glass vial from breaking during centrifuge, if necessary, let sample settle for 2-5 minutes and transfer 2 mL of sample to a clean 4 mL glass vial or similar, then centrifuge. Also, make sure the centrifuge rotator is balanced by using equal amounts of sample on each end of the rotator.).
- 5.5 Dilute sample 20-fold by adding 50 µL of sample to 950 µL ABRAXIS® Imidacloprid Sample Diluent (included in ABRAXIS® Imidacloprid ELISA kit. Additional buffers can be ordered).
- 5.6 The sample is ready for analysis, see ABRAXIS® Imidacloprid ELISA user's guide Section F. Assay Procedure.

6. Evaluation of Results

- The extracted sample assay results must be multiplied by a factor of 200 to account for the extraction dilution.
- Assay result (ppb) x dilution factor = concentration of Imidacloprid in sample.
- Assay results showing a lower concentration than Standard 1 (0.075 ppb) are considered to be negative. Assay results containing a higher concentration than Standard 6 (1.2 ppb) must be diluted to obtain accurate results.

7. For ordering or technical assistance contact:

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