Chloramphenicol: Alternate Shrimp Extraction Protocol

1. Intended Use
For the extraction of Chloramphenicol from shrimp using ethyl acetate and hexane.

2. Range of Detection
The range of detection is 0.025ppb to 2ppb in matrix. If samples exceed calibration, are known to contain higher analyte levels, or a higher detection range is necessary, samples can be diluted.

3. Materials Required (Not Provided)
Pipette(s) capable of delivering 20uL-200uL and 100uL-1,000uL
Glass vials with Teflon-lined caps
50mL conical tubes
De-ionized or distilled water
Ethyl Acetate (HPLC grade)
Hexane (Practical grade)
Nitrogen gas
Evaporation block capable of reaching 50ºC
Vortex mixer
Overhead mixer or shaker
Centrifuge capable of 3,000 x g
Food processor or blender
Scoopula or disposable, wooden tongue depressors

4. Notes and Precautions
4.1 Shrimp must be thawed and de-shelled.

5. Procedure
5.1 Homogenize a sufficient amount of thawed and de-shelled shrimp (e.g. 10g) with a food processor or blender.
5.2 Weigh 3g of homogenized sample into a 50mL conical tube.
5.3 Add 3mL of de-ionized/distilled water.
5.4 Add 6mL of ethyl acetate.
5.5 Vortex tube briefly then place on an overhead rotator or shaker for 10 minutes.
5.6 Centrifuge tube for 10 minutes at 3,000 x g at room temperature (23ºC).
5.7 Transfer 4mL of the supernatant (ethyl acetate), which should be clear in color, into a 4mL glass vial.
5.8 Evaporate the supernatant under a stream of nitrogen at 50ºC.
5.9 Add 1mL of hexane to the dried residue in the 4mL glass vial and vortex for 1 minute.
5.10 Add 0.5mL of sample buffer and vortex the 4mL glass vial for 1 minute.
5.11 Centrifuge the 4mL glass vial for 10 minutes at 3,000 x g at room temperature.
5.12 Transfer all of the top layer (hexane) into a solvent waste container. A small amount ($\leq$ 200uL) of the bottom layer (sample buffer) maybe removed with the hexane if necessary to ensure removal of the entire hexane layer (hexane will interfere in the ELISA—producing inaccurate results).
5.13 Dilute 100uL of the bottom layer into 300uL of sample buffer.
5.14 Sample is ready for analysis. Proceed to Section C of the Abraxis Chloramphenicol user's guide.

6. Evaluation of Results
6.1 The ELISA result will show the Chloramphenicol concentration contained in the shrimp samples (no correction factor is necessary). Highly contaminated samples (those outside of the calibration range of the assay) must be diluted and re-analyzed.
6.2 See Section D of the Abraxis Chloramphenicol user's guide for data-evaluation assistance.

7. Assistance

For ordering or technical assistance contact:
Abraxis LLC
54 Steamwhistle Drive
Warminster, PA 18974
Tel.: (215) 357-3911
Fax: (215) 357-5232
Email: info@abraxiskits.com
Web: www.abraxiskits.com