SPATT Set

Catalog Number: CO2029

Kit Contents:

Component		Amount	Storage Condition
Embroidery hoops 3”		1	RT
SPATT bags	• 3g HP20 resin • 8x4inch plankton mesh	3	4°C In packaging or in new closed container with resin submerged in ultrapure water
Zip ties		1	RT
Disposable Scoopers		1	RT

Introduction

Solid Phase Adsorption Toxin Tracking (SPATT) is a biomimetic *in-situ* water monitoring tool that falls under an expanding umbrella of passive samplers. It serves to warn researchers of toxin-producing harmful algal bloom (HAB) developments early on. It has been popularized through its affordability, ease of use, and its ability to capture ephemeral events in marine, brackish, and freshwater environments. Its uptake of contaminants has been shown to be more similar than other sampling methods to that of aquatic species like bivalves, mussels, and clams. It provides an average bioavailable fraction of a toxin over deployment time that can be used to determine an overall toxin risk to organisms. The sampling period typically depends on the bioactivity at a site, ranging from 24 hours to 4 weeks in most cases.

A SPATT passively absorbs and desorbs extracellular compounds over its stretch of time at a sampling site; in an organism, a toxin would go through biochemical detoxification processes. Passive samplers have a higher sensitivity for more compounds and provide improved stability and preservation of these compounds within the resin. SPATT devices capture less commonly detected cyanotoxins (e.g. cylindrospermopsin) at lower concentrations than that of a grab sample (collected at one point in time). Grab samples are limited in scope and sensitivity, and underrepresent toxins like microcystin-LR, which is picked up very reliably through SPATT technology.

Attogene’s SPATT Set includes 3 ready-to-use SPATT bags with pre-activated HP20 resin held by plankton mesh, a 3” embroidery hoop that can be reused at your convenience, and a disposable scooper to handle the resin for analysis. The SPATT Set is inspired by the works of Dr. Meredith Howard and Dr. Raphael Kudela, who produced a standard operating procedure for the device in 2018[a]. Passive sampling is an essential component of a comprehensive monitoring strategy, and our SPATT is a convenient, economical way for state and private agencies to conduct routine, proactive water quality control tests. It is well-founded that an integrated approach to cyanotoxin monitoring is the best way to keep water safe for human, farm animal & pet use.

(a) Howard, M.D.A.; Hayashi, K.; Smith, J.; Kudela, R. and Caron, D. (2018) Standard Operating Procedure for Solid Phase Adsorption Toxin Testing (SPATT) Assemblage and Extraction of HAB Toxins. University of California and University of Southern California, 14pp. DOI: dx.doi.org/10.17504/protocols.io.xkpfkvn

- Deployable in harsh conditions
- Easy, fast, simple, affordable, reliable
- Detection marker of toxin contamination in shellfish and bioaccumulation overall
    - Impacts ecological and public health
- Can be used as an early warning system for bloom events
- Ready-to-use with pre-conditioned ("activated") HP20 resin; no initial methanol & wash steps
- High enrichment of lipophilic and hydrophilic contaminants
- Mouse bioassay alternative, and bivalve sampling supplement

Resin

Specifications:

Product: Diaion Resin, HP20
Type: Synthetic Adsorbents
Matrix: Styrene-DVB, Porous, Rigid cross-linked polystyrene/divinylbenzene matrix
Characteristics: Nonpolar, aromatic
Water Content: 55 – 65%
Particle Size Distribution thru 250µm: 10% maximum
Effective Size: 0.25 mm minimum
Specific Surface Area: 90 m^2/g
Pore Volume: 1.3 mL/g
Pore Radius: 290 Å

Recommended Conditions:

Maximum Operating Temperature: 130 °C
Operating pH Range: 0 – 14
Minimum Bed Depth: 800 mm
Flow rate (BV/h)
Loading: 0.5 - 5
Displacement: 0.5 - 2
Regeneration: 0.5 - 2
Rinse: 1 - 5

Advantages to the HP20 were also identified as the best resin for use with lipophilic toxins in seawater for prolonged (days) deployment, with reasonably linear uptake and a combination of good adsorption and desorption capabilities. Other resins performed better under some circumstances but were found not to be as universally applicable to a broad range of toxins, deployment times, and recovery methods (Zendong et al. 2014). Thus, there is growing acceptance of HP20 resin as a "universal" SPATT resin, with the best overall combination of characteristics.

Used to capture:

- Cyanotoxin (e.g. microcystin and cylindrospermopsin)
- Saxitoxin & derivatives (GNTXs, C-toxins), and other paralytic shellfish toxins (PSTs)
- Domoic acid (DA)
- Nodularin
- Anatoxin-a
- Brevetoxins (via "red tide"-causing dinoflagellate *Karenia brevis*)
- Okadaic acid (OA) & derivative Dinophysistoxins (DTXs) and other Diarrheic shellfish poisoning (DSP) toxins
- Yessotoxin (YTX) and Pectenotoxins (PTXs)
- Cyclic imines (CIs), e.g. Spirolides (SPXs), Gymnodimines (GYMs), Pinnatoxins (PnTXs)
- Ciguatoxin (CTX) & precursor Gambiertoxin (GTX), Maitotoxin (MTx), and other ciguatera fish poisoning (CFP) toxins

Materials Not Provided:

Component
Weighted line, cannister or structure (e.g. stake)
Methanol
Ultrapure, Milli-Q water (MQ)
Filter Manifold
Disposable Chromatography Column, 20mL bed
Glass scintillation vials, 20 mL

Handling and Storage – upon receipt

Do not freeze before use.

1. Pour the water out, refill the beaker, and repeat rinses until the temperature of the water does not increase when the hoop is placed in the beaker

The SPATT bags will arrive in a heat-sealed foil bag with ultrapure water covering the resin to ensure the resin does not dry out. Do not open this unless you intend to place the SPATT in a new sealed cannister/bag of choice (this can be a Ziplock).

3. Store your SPATT Set in a refrigerator until deployment. HP20 resin is stable for months in a refrigerator.

Deployment:

1. Use a zip tie to attach SPATT to a structure or a weighted line.

Handling and Storage – from the field

1. Upon collection rinse as much silt and debris from the flexi-hoop ring or 'tea-bag' as possible using field water.
2. Put SPATT bag into a labeled ziplock bag (does not need to be in water). Writing with sharpie pens directly onto the ziplock bag is recommended.
3. Freeze immediately at < -4°C until your extraction of toxins in the lab.

More information on SPATT device and the extraction of microcystins and other water contaminants can be found in the references section of the Attogene website, one of them being the SOP produced by Drs. Howard and Kudela.

Extraction

This extraction process described is for the analysis of both marine toxins and cyanotoxins.

Summary of recommended extraction protocols for SPATT based on the toxins of interest.

Toxins	Extraction	Amount	Methodology
Cyanotoxins, Okadaic acid, Saxitoxin and related PSTs	50% MeOH in MQ water	10mL	Kudela, 2011
	50% MeOH in MQ	20mL	
	50% MeOH in MQ	20mL	
DA and Cyanotoxins	50% MeOH in MQ	10mL	Lane et al., 2010; Peacock et al., 2018
	1M ammonium acetate in 50% MeOH	10mL	
	1M ammonium acetate in 50% MeOH	20mL	
Anatoxin-a[§]	100% MeOH with 2% formic acid or *trifluoroacetic acid (TFA)*[¥]	10mL	Kudela, unpublished *TFA: Azevedo et al., 2011*
	50% MeOH in MQ	20mL	
	50% MeOH in MQ	20mL	

§ This protocol is for anatoxin-a specifically, and at the time of this writing, the recovery effects on other toxins from this extraction are being evaluated; Microcystin recovery is not affected (R. Kudela, unpublished data).
¥ Using 100% MeOH with 2% formic acid or TFA for the first extraction improves recovery for anatoxin. This first extract should be diluted back to 50% MeOH before analysis on the LCMS.

Notes with Passive Sampling: There are some challenges when deploying passive samplers that primarily originate from environmental variables occurring during the deployment period. Water turbulence affects the diffusion of the compound(s) from water to the receiving sorbent. Similarly, biofouling affects transfer and limits diffusion because biofilms increase thickness and block the pores of the membrane and the sorbents. Water turbulence may also move a PSD from its original location or wash it away altogether, making it difficult to recover the equipment. In addition, the equipment is exposed to vandalism and theft.
It is important to add to research of sampling rate (Rs) values for a wide range of compounds to calculate time-weighted averages (TWA).
To estimate the TWA concentration of compounds, PSD calibration is required before deployment to determine the sampling rate (Rs) for the class of compounds or each target compound. The sampling rate is significantly affected by fluctuating environmental variables such as water temperature, pH, flow velocity, and biofouling. These factors are site-specific and affect the estimation of Rs and subsequent compound concentration estimates in water. Calibration can be conducted in the laboratory or field, as needed. A lab calibrated Rs is commonly used because it is easier, cheaper, and water parameters such as pH, conductivity, and temperature are controlled. Moreover, a large list of calibration data developed in laboratories is available. For the Water Boards to further refine these Rs lists for constituents of emerging concern and legacy contaminants, a special Rs study must be conducted. A draft study plan is provided in the appendix. USGS (2010) has developed Rs list and a tool to calculate TWA for hydrophilic compounds. California Water Resources Control Board (2021)