SPATT Set

Catalog Number: CO2029

Kit Contents:

Component		Amount	Storage Condition
Embroidery Hoop		1	RT
SPATT Bag	• 3g HP20 resin • 100 micrometer plankton mesh	3	4°C In packaging (comes with ultrapure water / 8% ethanol) or in new closed container with resin submerged in ultrapure water
Zip tie		1	RT
Disposable Scooper		3	RT

Introduction

Solid Phase Adsorption Toxin Tracking (SPATT) is an *in-situ* biomimetic 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 major 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 4" embroidery hoop that can be reused at your convenience, and disposable scoopers 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], then modified it in 2020. Passive sampling is an essential component of a comprehensive monitoring strategy, and our SPATT is a convenient, economical way for government 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

SPATT

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

HP20 Resin

HP20 resin has been identified as the most "universal" resin for use with lipophilic and hydrophilic toxins in water for prolonged deployment, with efficient linear uptake. Other resins performed better under some circumstances but were found not to be as universally efficient with a broad range of toxins, deployment times, and recovery methods (Zendong et al. 2014).

Used to Capture:

- Cyanotoxin (e.g. microcystin and cylindrospermopsin)
    - MC-LY has over 15% recoveries than HLB resin, measured with triplicate SPATT data (Kudela 2020 Extraction Protocol with HP20 resin)
    - MC-LR and MC-WR had the highest recoveries overall
- Saxitoxin & derivatives (GNTXs, C-toxins), and other paralytic shellfish toxins (PSTs)
- Nodularin
- Okadaic acid (OA) & derivative Dinophysistoxins (DTXs) and other Diarrheic shellfish poisoning (DSP) toxins
- Anatoxin-a
- Brevetoxins (via "red tide"-causing dinoflagellate *Karenia brevis*)
- Yessotoxin (YTX) and Pectenotoxins (PTXs)
- Domoic acid (DA)
- 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:

Purpose	Component
General PPE	Gloves
Deployment	Weighted line, cannister or structure (e.g. stake)
Storage, Extraction	Ultrapure, Milli-Q water (MQ)
Extraction	Methanol and other eluents
	Filter Manifold
	Disposable Chromatography Column, 20mL bed
	Glass Scintillation Vials, 20 mL

Handling and Storage – upon receipt

Immediately place your SPATT Set in a refrigerator until deployment.

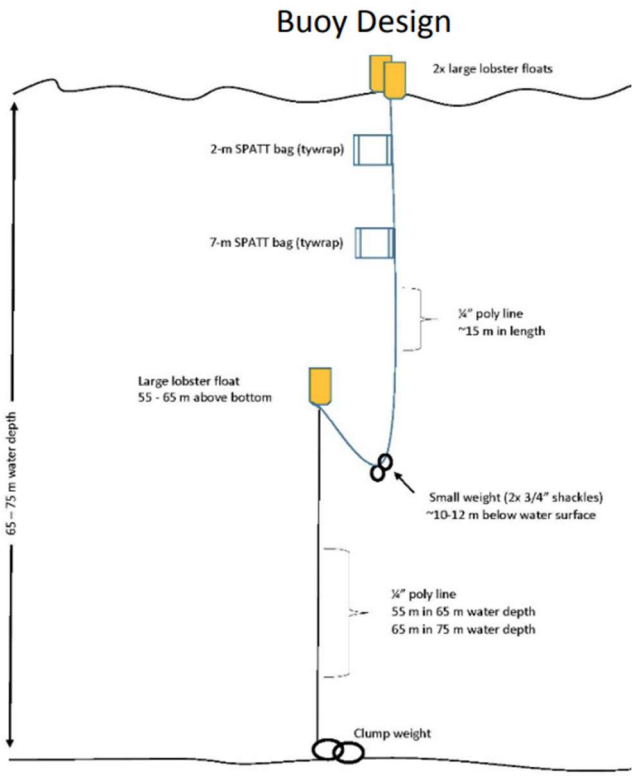
Do not freeze before use. The SPATT bags will arrive in an airtight bag with ultrapure water and 8% ethanol (C_2H_6O), covering the resin to ensure it does not dry out. Do not open this before sampling unless you intend to place the SPATT in a new sealed reservoir of choice, where resin is soaking in deionized water. This can be a Ziplock bag.

- If the resin dries at all before sampling, it needs to be "re-activated" with 100% methanol or ethanol..
- For storage longer than a month, contact Attogene for extended storage recommendations.

Deployment:

Assemble your SPATT device. Use the zip tie to attach the SPATT to structures such as a pier, piling, floating platform, buoy, or a weighted line. One can also use cages or cannisters typically used for POCIS™ deployment. SPATT can be attached directly to a buoy, or more elaborate designs can be configured (see right).

Ensure that the sampling site is at least 1 meter in depth.



Buoy Design by George Robertson (CA, Orange County Sanitation District)

Safety:

While handling the SPATT bags, it is best practice to wear fresh gloves. Use of gloves and proper PPE is required again during all extraction steps, and if re-activation of resin is necessary (handling 100% methanol or ethanol). The SPATT bags are shipped in a small percentage of ethanol– placing them directly into the field is safe for the environment, as ethanol readily degrades in water.

Handling and Storage – in the field

1. Collect SPATT devices with gloves on. Rinse as much debris from the embroidery hoop and mesh bags as possible using field water.

3. Remove bags from the hoop by loosening the metal tension screw, and place SPATT bags into a labeled ziplock bag (does not need to be in water) individually or grouped by site. Writing with sharpie pens directly onto the ziplock bag is recommended. Essential information to note: Site name, date/time deployed, date/time retrieved, and observations.

4. **Freeze immediately on ice**. If not moving immediately to extraction steps, place SPATT bags in freezer at ≤ -20°C as soon as possible. This maximizes toxin recovery, and the resin is much easier to manipulate when frozen.

Extraction

Summary of recommended extraction protocols for SPATT based on the algal toxins of interest, with volumes based on 3g of resin.

Note: This table is based on current published protocols, but extraction methodologies can be customized to your target analytes and sample matrices.

Toxins	Extraction	Volume	Methodology
Domoic Acid	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	
All algal toxins	100% MeOH, acidified with 2% formic acid	10mL	Kudela, unpublished
	50% MeOH in MQ	20mL	
	50% MeOH in MQ	20mL	

Notes on Passive Sampling

SPATT Uptake Factors

- **Water turbulence** : Affects the compounds' diffusion from water to resin
- **Biofouling and biofilms** : Can cause resin pore blockage

Water temperature, pH, flow velocity, and biofouling all are integral factors of a SPATT's sampling rate, which comes into play at the beginning when you calibrate and place the SPATT bag(s).

Mindful SPATT Placement

This is important as you need for the sampling site to be at least 1 meter in depth, and a SPATT's placement can be affected by foot traffic (people can be suspicious of the deployed SPATT setup and remove it) and water currents may remove the SPATT from its original placement.

Other Notes for Interpretation

- SPATT devices only measure dissolved toxins, not total toxins; extracellular not intracellular.
- A SPATT's 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 compound concentration estimates in each corresponding water sample. Without calibration methods, the SPATT becomes more of a screening tool than a tool for standardized quantification.

Resin Specifications

Product: Diaion Resin, HP20
Type: Synthetic Adsorbents
Matrix: Styrene-DVB, Porous, Rigid cross-linked polystyrene/divinylbenzene matrix
Characteristics: Nonpolar, aromatic
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

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