## Relevance of Okadaic Acid Determination

Diarrhetic shellfish toxins (DSTs), including okadaic acid (OA) and its analogues dinophysistoxins-1 and -2 (DTX1 and DTX2), are marine lipophilic toxins produced by certain dinoflagellates during harmful algal blooms (HABs). These toxins are the causative agents of Diarrhetic Shellfish Poisoning (DSP) in humans. Consumption of DST-contaminated bivalve mollusks can cause gastrointestinal symptoms, including diarrhea, nausea, vomiting, abdominal pain, and cramps. Due to these public health risks, regulatory authorities worldwide, including the United States, Canada, and Europe, require routine monitoring of bivalve shellfish. A maximum regulatory limit of 160 µg okadaic acid equivalents per kg of shellfish tissue is enforced for harvest closures and market access control.

## Validation Data

| | |
|---|---|
| Test Sensitivity and Threshold: | DSP Alert™ is engineered with a conservative decision threshold of 135 µg OA equivalent per kilogram of shellfish tissue (135 ppb) to provide an added safety margin relative to the regulatory limit. |
| Selectivity: | The assay detects OA, DTX1 and DTX 2. |
| Comparability with LC-MS/MS: | In single-laboratory validation studies, the assay demonstrated 100% agreement with standard LC-MS/MS reference methods for samples containing DSP toxins at or above the regulatory action level (160 µg OA eq/kg) in mussels and scallops. No false negatives were observed at or above the regulatory limit. |
| General Limited Warranty: | The DSP Alert™ Rapid Test Kit is intended for research and pre-testing purposes only and is not a replacement for officially validated regulatory methods. **Users are responsible for determining product suitability. Sensoreal Inc. provides no expressed or implied warranties and is not liable for indirect, incidental, or consequential damages.** |

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# Sensoreal® DSP Alert™ Rapid Test for detection of DSP toxins in Shellfish (Centrifuge)

Product ID: ALRT-DSP-KIT

## 1. General Description and Principle of the Method

Sensoreal DSP Alert™ is a rapid lateral flow immunoassay (LFA) designed for the qualitative detection of shellfish toxins which cause diarrheal shellfish poisoning (DSP), mainly okadaic acid (OA) in bivalve mollusks. The assay operates on a competitive binding format and has been evaluated on mussels, oysters, scallops, cockles and razorclams.

DSP toxins such as OA is extracted from homogenized shellfish tissue using a 100% methanol solution. The extract is then diluted and incubated with a lyophilized antibody–gold nanoparticle conjugate. If OA is present in the sample, it binds to the conjugate antibodies, reducing the amount of free antibody–gold complex available. The mixture is then applied to the lateral flow cassette and migrates across a membrane containing immobilized OA-protein at the test line. Consequently, the test line intensity is inversely proportional to the OA concentration (higher OA concentration = fainter test line). A control line will appear in all valid tests to confirm proper assay function. Results are objectively interpreted using the Sensoreal SensoRead™ scanner.

## 2. Safety

Treat all samples, extracts, and used test components as potentially contaminated with DSP toxins and biological material. Wear appropriate PPE, including gloves, safety goggles, and a laboratory coat. The DSP Alert™ protocol uses 100% methanol for extraction, which is hazardous and flammable, and includes a hydrolysis step with 2.5 M sodium hydroxide (NaOH) and 2.5 M hydrochloric acid (HCl) to release esterified DSP toxins. Handle all solvents and reagents in a well-ventilated area, follow institutional chemical safety procedures, and keep away from ignition sources.

## 3. Storage

-Lateral Flow Test Strips (Cassettes): Store at the temperature specified on the label. Do not freeze. Keep cassettes in their original, sealed pouches until immediately prior to use to protect them from moisture and humidity.
-Antibody Conjugate Vials (Lyophilized): This is the only kit component that may be frozen. Store frozen or as strictly specified on the product label. Keep vials sealed and do not open until ready to perform the assay.
-Diluent Powder Vials: Store at the temperature specified on the label. Do not freeze. Keep sealed to protect from excess heat and moisture.
-Buffer Solution: Store as directed on the label.
**-General Stability:** Protect all kit components from excess heat and direct sunlight. Do not utilize kit components past their printed expiration date or if they exhibit any signs of damage or deterioration.

## 4. Procedural Notes

-The DSP Alert™ test is optimized for analyzing freshly harvested bivalves, intact samples refrigerated for under 48 hours, or previously homogenized tissue kept frozen. Prior to beginning the extraction process, it is critical that all chilled or frozen samples are allowed to fully equilibrate to ambient room temperature.
-Unprocessed shellfish can be safely held under refrigeration (2–8°C) provided they will be tested within two days of harvest. If the testing timeline extends beyond this 48-hour window, the shellfish meat must be completely shucked, homogenized (as outlined in Section C), and stored at -20°C to ensure toxin stability prior to extraction.
-Please note that this protocol is exclusively validated for bivalve shellfish matrices. Any application to alternative sample types requires thorough independent validation by the user before routine testing is implemented.
-Follow the specified extraction, dilution, incubation, and read times exactly as detailed in Section C, D and E. Deviations from the protocol may adversely affect test performance.
- To avoid cross-contamination, use clean, disposable pipette tips and fresh extraction/dilution materials for each individual sample.
-Do not open the lateral flow cassettes or conjugate components until ready to initiate testing.
-Both visual and scanner-based interpretation are supported. Users may utilize the designated Sensoreal SensoRead™ scanner and its corresponding settings, or perform a manual visual interpretation of the results.
-Dispose of sample extracts, methanol-containing waste, and used kit components in strict accordance with institutional, local, and regulatory environmental requirements.
-As with any screening technique, positive results leading to regulatory action or market closure should be confirmed by a recognized alternative quantitative method (e.g., LC-MS/MS).

# Protocol Instructions

## A. Kit Components

The DSP Alert™ kit includes the following components:

1. Lateral Flow Test Strips (Cassettes)
2. Conjugate Vials (lyophilized antibody-gold nanoparticle)
3. Diluent Powder Vials ("D")
4. Buffer Solution ("B")

## B. Materials and Equipment Required (Not Provided)

1. Sensoreal's SensoRead™ Scanner (used for result interpretation)
2. Shellfish shucking tools (knife, etc.)
3. Blender for tissue homogenization, Analytical weighing balance, Thermometer, Electronic timer
4. Centrifuge (compatible with 15 mL conical tubes and capable of 5000 rpm)
5. Adjustable pipettes covering volumes of 20–200 µL and 100–1000 µL and disposable pipette tips
6. 15 mL conical tubes (for extraction and collection), 2 mL microcentrifuge tubes, 5 mL glass vials with screwcap lid (for heating and hydrolysis step)
7. Water bath or Dry Block Heater
8. 100% Methanol, Sodium hydroxide (NaOH) pellets, Hydrochloric acid (HCl): 37% w/w, Deionised (DI) water
9. The use of test tube racks and a vortex mixer is recommended for operational ease; however, these components are not mandatory for the methodology.

## C. Reagent Preparation, Sample Collection, and Processing

1. Preparation of 2.5M NaOH: Take 1g of NaOH pellets and dissolve in 10 mL of DI water in 15 mL conical tube. Shake vigorously to ensure all pellets dissolve.
2. Preparation of 2.5M HCl: Pour 7.9 mL of DI water in 15 mL conical tube. Carefully and slowly add 2.1 mL of 37% w/w HCl.
3. For each sample ID, shuck a representative population of 10 to 12 individual animals to obtain a minimum of 100 g of pooled shellfish tissue.
4. Thoroughly rinse the exterior of the shellfish with deionized or distilled water to remove extraneous debris, such as sand or mud.
5. Transfer the collected tissue to a sieve and allow it to drain to remove excess fluid.
6. Homogenize the tissue using an immersion blender (or equivalent mechanical blender) until a completely uniform, smooth consistency is achieved.

## D. Toxin Extraction and Hydrolysis

1. Using a clean spatula and weighing balance, weigh exactly 2.5 g of the homogenized shellfish sample and transfer it into a 15 mL conical tube.
2. Add 10 mL of 100% methanol extraction solvent to the conical tube containing the sample.
3. Cap the conical tube tightly and shake vigorously (or utilize a vortex mixer) for 5 minutes.
4. Transfer the tube to a centrifuge and spin at 4000 rpm for 5 minutes to separate the particulate matter from the toxin-containing solvent.
5. Following centrifugation, carefully transfer the supernatant (the clear liquid) into a clean 15 mL conical tube.
6. Preheat a water bath or Dry Block Heater to **76°C**. Use a thermometer to monitor the temperature and ensure it stabilizes before proceeding.
7. Using clean pipettes, transfer 800 µL of the supernatant in a 5 mL glass vial. Add 100 µL of 2.5M NaOH solution. Close the screw cap, shake well and place into the water bath or Dry Block Heater. Heat at 76°C for 40 minutes. This is the hydrolysis step.
8. After 40 minutes, take out the glass vial from the water bath/Dry Block Heater and allow the contents to cool to room temperature. To the cooled solution, add 100 µL of 2.5M HCl. This will stop the reaction. This is the hydrolyzed extract.

## E. Test Strip Sample Analysis Procedure

*Ensure the Sensoreal SensoRead™ scanner is turned on and the correct lot QR code has been scanned prior to initiating the test.*

1. Locate the Diluent powder tube ("D"). Using a pipette fitted with a new tip, add 2 mL of Buffer "B". Shake vigorously until all powder dissolves. This is the diluent solution. With fresh pipette tips, transfer 800 µL of diluent solution into a new 2 mL microcentrifuge tube. To this, add 200 µL of the hydrolyzed extract. Mix thoroughly. This is the Diluted Sample.
2. Using a pipette fitted with a new tip, transfer 70 µL of the Diluted Sample into the Conjugate vial. Pipette the mixture up and down to achieve complete dissolution of the lyophilized pellet, ensuring that no red specks remain adhered to the interior walls of the tube. Once the pellet is fully dissolved and the solution is homogeneous, let the vial sit uninterrupted at room temperature for exactly 10 minutes.
3. After the 10-minute incubation, transfer the contents of the Conjugate mix into the sample port of the test cassette.
4. Leave the cassette on a flat, horizontal surface. Set a timer and allow the assay to develop for exactly 10 minutes.

## F. SensoRead™ Scanner Analysis

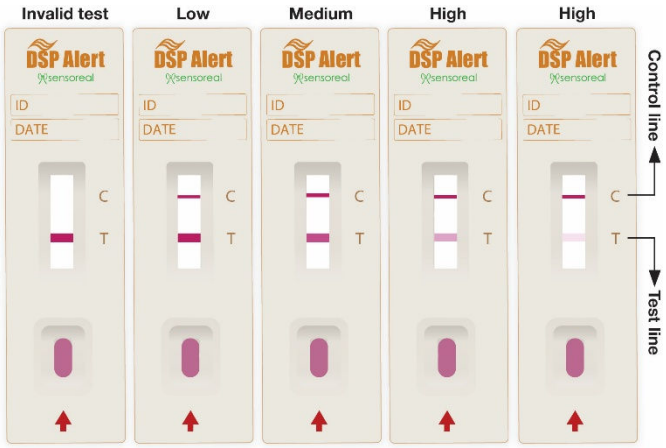
1. After exactly 10 minutes of development, immediately insert the test cassette into the scanner slot of the SensoRead™ device. Follow the on-screen prompts: Select Cassette -> DSP Alert
2. You will be prompted to identify the batch being used. Either select an existing Lot from the on-screen list, or for a new Lot, press "Add New Lot" and scan the batch-specific QR code located on the LFA test pouch or the Certificate of Analysis. This QR code provides the system with integral calibration parameters required for accurate interpretation. *(Note: Each Lot only needs to be scanned once; for subsequent tests with the same Lot, simply select it from the saved list).*
3. Once the Lot is confirmed, enter the unique Sample ID using the on-screen keypad and press "Run Test". The device will immediately initiate the analysis process to receive the qualitative result (T/C ratio and classification).

## G. Interpretating the Results

Assay results can be interpreted visually or digitally using the SensoRead™ scanner, which calculates the Test-to-Control (T/C) line intensity ratio. While the absolute intensity of the control line may fluctuate between runs, a visible control line must be present for the assay to be considered valid; the absence of a control line indicates an invalid result requiring a retest.

The SensoRead™ scanner provides an objective determination of line intensities for consistent interpretation, as well as a digital photographic record of all test strips.

-High: The scanner has detected OA and other DSP toxins at or above the 135 µg OA eq/kg threshold. The scanner will flag this sample, indicating it has reached or exceeded the precautionary limit.
-Medium: The T/C ratio indicates concentrations between 80-135 µg OA eq/kg threshold, and the sample is considered compliant.
-Low: The T/C ratio indicates concentrations below 80 µg OA eq/kg, and the sample is considered compliant.




| Test Line | Control Line | Interpretation |
|---|---|---|
| Present | Absent | Invalid Test (Repeat Test) |
| Faint or Non-Visible | Visible | High: ≥ 135 µg OA eq/kg |
| Moderate Intensity | Visible | Medium: 80-135 µg OA eq/kg |
| Equal to or More Intense than Control Line | Visible | Low: < 80 µg OA eq/kg |

*Note: It is essential to perform the reading at the designated 10-minute mark. Determinations made using strips that have dried for more or less than the required time may be inaccurate, as chromatographic line intensities vary with drying time. To ensure a rigorous safety margin against the 160* µg OA eq/kg *NSSP regulatory limit, the DSP Alert™ system is intentionally calibrated to a conservative early-warning threshold of 135* µg OA eq/kg.

For supplemental technical data or methodological assistance, please direct all inquiries to Sensoreal Technical Support at sales@sensoreal.com.