Product 9561

Read instructions carefully before starting test

Reveal® 2.0 for DSP

For use with approved Neogen reader

Store at 18–30°C (64–86°F) • Do not freeze

THE TOXINS

Toxins that cause diarrhetic shellfish poisoning (DSP) include the okadaic acid (OA) group of toxins. OA is produced by marine dinoflagellates such as *Dinophysis*, and has structural analogs referred to as the dinophysistoxins (DTXs). Clinical toxicological effects attributed to DSP following consumption of contaminated seafood include diarrhea, nausea and vomiting. Human cases have been reported since the early 1960s in Norway and elsewhere on a global scale. The established European Union maximum permitted levels are 160 µg OA equivalents (OA, DTXs, pectenotoxins) per kg shellfish meat (160 ppb). The U.S. Food and Drug Administration action limits are 160 µg (160 ppb) OA equivalents (OA, DTXs) in shellfish.

INTENDED USE

Reveal® 2.0 for DSP is an immunochromatographic lateral flow assay used for the rapid and practical qualitative analysis of shellfish possibly contaminated by OA group toxins (OA, DTX-1, DTX-2, and DTX-3). The test can detect as little as 160 ppb of OA equivalents in shellfish samples.

INTENDED USER

The test kit is designed for use by quality control personnel and other personnel familiar with handling shellfish possibly contaminated by OA toxins.

ASSAY PRINCIPLES

Reveal 2.0 for DSP is a single-step lateral flow device based on a competitive immunoassay format. In summary, the shellfish extract is wicked through a reagent zone, containing antibodies specific for OA-group toxins that have been conjugated to colored particles. If the toxins are present in the sample, the toxin will be captured by the particle-antibody complex. The complex then is wicked onto a membrane, which contains a stationary capture zone of a toxin-protein conjugate. This zone captures any uncomplexed toxin particle-antibody. Therefore, as the concentration of toxins in the sample increases, the test line intensity decreases. The membrane also contains a stationary control zone that will always form regardless of the level of toxins.

STORAGE REQUIREMENTS

Store kit components at room temperature (18–30°C, 64–86°F) to ensure full shelf life. Test strips should remain capped in their original sample tubes until used to ensure optimal performance.

MATERIALS PROVIDED

Reveal 2.0 for DSP (Neogen item 9561)

1. 24 Reveal 2.0 for DSP lateral flow test strips
2. 24 wells
3. 24 vials of DSP buffer A (clear cap)
4. 24 vials of DSP buffer B (red cap)
5. 25 extraction bags
6. 48 disposable 100 µL pipettors

MATERIALS RECOMMENDED BUT NOT PROVIDED

1. Marine Biotoxins Starter Kit (Neogen item 9563)
   a. Microwell holder
   b. 1 roller
   c. 1 bag clip (white clip and green straw)
2. DSP Hydrolysis Pack (Neogen item 9554)
   a. 2.5 M sodium hydroxide (NaOH) solution (5 mL)
   b. 2.5 M hydrochloric acid (HCl) solution (5 mL)
   c. 24 glass sample vials
3. Distilled water
4. Methanol (analytical grade recommended, VWR 20864.320)
5. Filter syringes (Neogen item 9420)
6. Sample collection cups with lids (Neogen items 9428, 9428B)
7. Blender (Neogen items 9493, 9477 or 9495)
8. Scale capable of weighing 0.5–400 g ± 0.1 g (Neogen item 9427)
9. Timer (Neogen item 9452)
10. Graduated cylinder, 50 mL (Neogen item 9447)
11. Sample collection tubes with caps, 5 mL (Neogen item 9421, 9421B)
12. Pipettor, 100–1000 mL, and pipette tips (Neogen item 9463, 9464)
13. Heater block (capable of holding 76 ± 2°C (recommended VWR 460-3250 & 460-3280)
14. AccuScan Pro reader (Neogen item 9565) or Raptor SOLO Integrated Analysis Platform (Neogen item 9696)
15. Raptor cartridges (Neogen item 9681)
16. Raptor Exact Volume Transfer Pipettes (Neogen item 9682)

PRECAUTIONS

1. The test strips must remain inside the stay dry tube before use.
2. Store test kit at room temperature (18–30°C, 64–86°F) when not in use. Do not freeze.
3. Do not use kit contents beyond expiration date.
4. Treat all liquids, including sample extract, and used components as if contaminated with toxin. Gloves and other protective apparel should be worn at all times.
5. To avoid cross-contamination, use clean pipettors, extraction bags and fresh extraction solutions for each sample.

ACCUSCAN PRO READER SET UP

1. Enter the lot-specific QR code by selecting the QR code icon on the reader. Place the QR code into the cartridge and insert the cartridge into the reader.
   NOTE: For instructions on manually entering sample IDs, see the AccuScan Pro user manual.
2. Return to the home screen and select the test strip icon. Touch the **Marine Biotoxins** category, then select the **DSP** test type.

SAMPLE PREPARATION AND PRELIMINARY EXTRACTION

The sample to be tested should be collected according to accepted sampling techniques.

1. Obtain a representative sample. Shell the samples.
2. Thoroughly rinse the samples with distilled or deionized water, and allow any excess water to drain.
3. Homogenize (e.g., blend, puree) the shellfish in a high-speed blender.
   NOTE: A good homogenate is essential in order to obtain an accurate result.
4. Number both sides of an extraction bag using a marker, so that one side is labeled "1" and the opposite side labeled "2."
   NOTE: The extraction bag contains a mesh filter which allows for partial filtration of the sample. All samples/solution should only ever be added to side "1."
5. Weigh out 2 g (± 0.1 g) of homogenized sample in the bottom of the extraction bag on side "1."
   IMPORTANT: Ensure the entire sample is at the bottom of the bag prior to next step.
6. Add 8 mL of analytical grade methanol to side "1" of the extraction bag containing the sample.
7. Ensuring that the sample and methanol remain in the lower half of the extraction bag, position and hold the green straw approximately half-way down from the top of the bag. Fold the upper edge of the bag over the green straw. Firmly clip on the white clip to prevent leakage of the sample.
8. Place the extraction bag on a firm surface and press the roller firmly on the sample extraction bag, pushing the roller back and forth for **30 seconds** to aid in obtaining a homogeneous sample extract.
9. Slide out the green straw and remove the white bag clip.
10. Remove the bag contents from side "2" into a suitable container (there may be small pieces of shellfish remaining on side "1"). Discard the used extraction bag.
11. Ensuring the sample extract is well mixed, pour into the barrel of a Neogen filter syringe until it is almost half full. Place the plunger on top, and filter sample extract into a collection tube.
    IMPORTANT: The filtered solution should be transparent/clear and not cloudy. Should the filter syringe block or should the filtered extract not be clear, pour the unfiltered contents into a fresh syringe filter to ensure a clear solution.

NOTE: The Hydrolysis Extraction Procedure that follows allows users to detect OA, DTX-1, DTX-2 and DTX-3. If you are in a region without requirements for DTX-3 detection, the alternative Screen Procedure is available.

HYDROLYSIS EXTRACTION PROCEDURE (TO DETECT OA, DTX-1, DTX-2, DTX-3)

1. Switch on heater block to 76°C.
2. Ensuring that the filtered extract is well-mixed, transfer 800 µL into a glass vial.
3. Add 100 µL of NaOH (2.5M) and cap tightly. Shake vigorously by hand for **30 seconds.**
4. Heat the vial in the heater block at 76°C for **40 minutes.**
5. After 40 minutes, remove the vial from the heater block and allow it to cool to room temperature. Alternatively, the sample vial can be placed in ice to cool faster.
6. Add 100 µL of HCl (2.5M) and cap tightly. Shake vigorously by hand for **30 seconds.**
7. Remove 100 µL of the sample extract and add into a vial of DSP buffer A (clear cap).
8. Proceed to **Test Procedure.**

SCREEN PROCEDURE (TO DETECT OA, DTX-1, DTX-2)

1. Remove 100 µL of the sample extract using a disposable pipettor* provided (or alternatively, by use of a standard pipettor), and add into a vial of DSP buffer B (red cap). *To use the disposable pipettors, firmly press the top bulb of the pipettor, insert the tip into the solution, slowly release the top bulb to draw up the sample extract. Excess volume (e.g., above 100 µL) will overflow into the lower bulb, ensuring 100 µL is ready to dispense. Press the top bulb firmly and release slowly to dispense. Discard the used pipettor.
2. Proceed to **Test Procedure.**

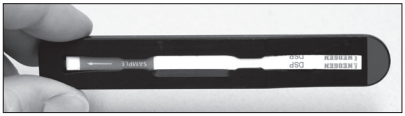
TEST PROCEDURE

1. Remove the appropriate number of microwells and place into the microwell holder.
2. Shake the vial containing sample extract and buffer vigorously by hand for **30 seconds.**
3. Remove 100 µL of the diluted sample extract and add to a microwell.
4. Remove the required number of DSP strips from the lateral flow stay dry tube and immediately close the tube.
5. Place the DSP strip with the sample end down into the well containing the filtered extract solution. Set a timer for **15 minutes** to allow the lines on the strip to develop.
6. After 15 minutes, remove the test strip and immediately interpret the results using AccuScan Pro reader.

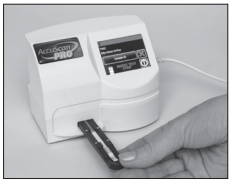
READING TEST RESULTS

Test strips should be read within **1 minute** of completion of the 15 minute incubation. Refer to **AccuScan Pro Reader Set Up** for test selection and set up information.

1. Fully insert the Reveal 2.0 for DSP test strip into the black R cartridge adapter with the sample end first and results facing out.



2. Insert the cartridge with test strip side up in the AccuScan Pro. The reader will automatically begin analyzing the cartridge. **CAUTION:** Removing cartridge prior to completion can result in invalid readings.
3. The AccuScan Pro reader will analyze the test strip and results will be displayed and stored in the reader.



NOTES

1. Ensure device is fully inserted into cartridge.
2. Readings should be made between **15–16 minutes**. Readings after 16 minutes may be inaccurate due to over-development of the device.
3. The strips must be read using a Neogen-approved reader.

TEST PROCEDURE – RAPTOR SOLO INTEGRATED ANALYSIS PLATFORM

1. Fully insert a Reveal for 2.0 for DSP test strip into a Raptor cartridge. Up to 3 strips can be inserted into the Raptor cartridge at one time to obtain duplicate or triplicate results.
2. Insert the Raptor cartridge containing the test strip(s) into the port within the Raptor SOLO Integrated Analysis Platform reader.
3. The bar code on the test strip(s) will be read — the Raptor SOLO reader identifies the type of test strip and the lot number.
4. If the lot number is not found in the system, the bar code reader on the front of the Raptor will turn on automatically.
5. Scan the QR code found on the side of the tube containing the test strips. The information will be stored on the system.
6. Enter Sample ID if desired.
7. Shake the DSP buffer vial (containing diluted sample) vigorously by hand for **30 seconds.**
8. Immediately transfer 400 µL of diluted sample into the Raptor cartridge using a new disposable pipettor.
9. The Raptor SOLO reader will start automatically.
10. Results will be displayed on the Raptor screen after the **15 minute** testing is complete.

PERFORMANCE CHARACTERISTICS

Reveal 2.0 for DSP is designed to screen for OA group toxins (OA and DTXs) in shellfish.

VALIDATED MATRICES

Mussels, scallops, oysters, clams and cockles.

NOTE: Neogen continues to validate new commodities. Please contact a representative for the latest validated commodity list.

CUSTOMER SERVICE

Neogen Customer Assistance and Technical Services can be reached by using the contact information on the back of this booklet. Training on this product, and all Neogen test kits, is available.

SDS INFORMATION AVAILABLE

Safety data sheets (SDS) are available for this test kit, and all of Neogen's test kits, on Neogen's website at foodsafety.neogen.com, or by calling Neogen at 800/234-5333 or 517/372-9200.

TERMS AND CONDITIONS

For Neogen's full terms and conditions, please visit www.neogen.com/en/terms-and-conditions.

WARRANTY

Neogen Corporation makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. If any materials are defective, Neogen will provide a replacement of the product. Buyer assumes all risk and liability resulting from the use of this product. There is no warranty of merchantability of this product or of the fitness of the product for any purpose. Neogen shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.

NOTES:

TESTING KITS AVAILABLE FROM NEOGEN

Natural toxins
• Aflatoxin, DON, ochratoxin, zearalenone, T-2/HT-2 toxins, fumonisin, histamine

Foodborne bacteria
• *E. coli* O157:H7*, Salmonella, Listeria, Listeria monocytogenes, Campylobacter, Staphylococcus aureus, Salmonella enteritidis*

Sanitation
• ATP, yeast and mold, total plate count, generic *E. coli* and total coliforms, protein residues

Food allergens
• Almonds, coconut, crustaceans, eggs, gliadin, hazelnut, milk, mustard, peanuts, sesame, soy, walnuts, multi-treenut

Genetic modification
• CP4 (Roundup Ready®)

Ruminant by-products
• Meat and bone meal, feed

Species identification
• Raw and cooked meat samples

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