

Okadaic Acid Lateral Flow Kit

Shellfish · Laboratory Use

Catalog Number: **AU2071**

For Research Use Only. Not for use in Diagnostic Procedures.

1. Introduction

Attogene's Okadaic Acid Lateral Flow Kit for shellfish is a rapid, single-use cassette test that screens bivalve shellfish tissue (oysters, mussels and similar) for okadaic acid (OA) at the regulatory limit of 160 µg/kg. Okadaic acid is extracted from homogenized tissue with 100% methanol, the extract is filtered and hydrolyzed with sodium hydroxide to release esterified okadaic acid, then neutralized, diluted in water, mixed with Sample Dilution Buffer and added to a cassette. The result is read at 30 minutes. Methanol, sodium hydroxide and hydrochloric acid are supplied by the customer. This version is made for the laboratory.

The test is a competitive immunoassay (Figure 1). As the sample flows along the strip it picks up colloidal gold coated with an anti-okadaic acid antibody. The test line (T) carries okadaic acid bound to BSA. If the sample contains no okadaic acid, the gold binds the test line and a red test line forms. Okadaic acid in the sample fills the antibody binding sites, so less gold binds the test line: the more okadaic acid in the sample, the weaker the test line. The control line (C), goat anti-mouse IgG, captures the gold that passes the test line and shows that the cassette ran correctly.

> **Important:** a weaker test line means MORE okadaic acid. Always read each sample against a negative control cassette run at the same time with an extract of okadaic acid-free shellfish of the same species, prepared in the same way as the samples (Sections 7 and 9).

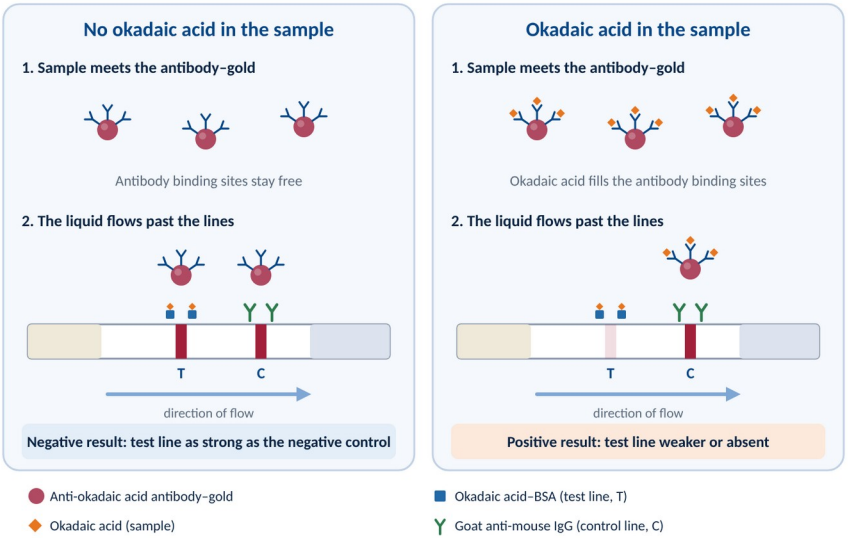


Figure 1. *Test principle. Left: with no okadaic acid, the antibody–gold binds the okadaic acid–BSA test line (T) and the excess binds the control line (C). Right: okadaic acid in the sample blocks the antibody, so the test line is weaker or absent; the control line still forms.*

About okadaic acid

Okadaic acid is a lipophilic polyether toxin produced by marine dinoflagellates of the genera Dinophysis and Prorocentrum. Together with its analogues, the dinophysistoxins, it causes diarrhetic shellfish poisoning (DSP). Okadaic acid inhibits the protein phosphatases PP1 and PP2A, which makes intestinal cells leak fluid and causes diarrhea, nausea, vomiting and abdominal pain. Filter-feeding shellfish concentrate the toxin by feeding on the algae, and part of it is converted to fatty acid esters (DTX3) in the shellfish. The regulatory limit in the United States and the European Union is 160 µg okadaic acid equivalents per kg of shellfish tissue (0.16 mg/kg).

Features & Benefits

- Screens at the regulatory limit: a positive result means okadaic acid at or above about 160 µg/kg.
- Methanol extraction with the supplied syringe and filter: no centrifuge needed.
- Hydrolysis step to release esterified okadaic acid.
- Results 30 minutes after the sample is added to the cassette, by eye or with a lateral flow reader.
- 5 samples per kit, each with its own negative control.

2. Kit Contents

Component	Quantity	Storage
Okadaic Acid Cassette, in a foil pouch with desiccant	10	Room temperature
Sample Dilution Buffer, 2X: 75 µL per tube	10 tubes	Room temperature
Syringe	10	Room temperature
Syringe filter, 1.2 µm	10	Room temperature
Manual	1	Room temperature

Kit size: 5 samples. Each sample uses two cassettes, two Sample Dilution Buffer tubes, two syringes and two filters: one set for the sample extract and one for its negative control extract. Methanol, sodium hydroxide and hydrochloric acid are not supplied (Section 5).

3. Product Specifications

Key specifications for experienced users and for setting up a lateral flow reader.

Parameter	Specification
Format	Single-use lateral flow cassette: sample port (S) and reading window, with C and T printed to the right of the window (Figure 2)
Assay principle	Competitive immunoassay: the test line weakens as okadaic acid increases
Reporter	40 nm colloidal gold conjugated to a mouse monoclonal anti-okadaic acid antibody
Test line (T)	Okadaic acid–BSA conjugate
Control line (C)	Goat anti-mouse IgG; captures gold that passes the test line
Samples	Homogenized bivalve shellfish tissue (whole edible tissue)
Extraction	1.0 g of homogenate + 4.0 mL of 100% methanol; 2 mL of the mixture filtered through a 1.2 µm syringe filter (about 1.5 mL recovered)
Hydrolysis	1.5 mL of filtrate + 100 µL of 2.5 M sodium hydroxide, 30 minutes at 76 °C; cooled; neutralized with 100 µL of 2.5 M hydrochloric acid
Dilution	Hydrolyzed extract diluted 3-fold in deionized water (100 µL + 200 µL)
Sample to cassette	75 µL of diluted extract mixed with 75 µL of 2X Sample Dilution Buffer; the entire tube (about 150 µL) goes to the sample port

Parameter	Specification
Methanol in the test	About 25% in the diluted extract and about 12% in the cassette
Concentration in the test	160 µg/kg in tissue corresponds to about 12 ng/mL in the diluted extract and about 6 ng/mL in the cassette if all the okadaic acid is extracted
Decision point (cutoff)	160 µg/kg (the regulatory limit). Positive: okadaic acid at or above about 160 µg/kg
Okadaic acid forms detected	Free okadaic acid, and okadaic acid released from its esters by the hydrolysis step. Cross-reactivity with DTX1, DTX2 and DTX3: to be determined
Time to result	Read at 30 minutes
Controls	Negative control: an extract of okadaic acid-free shellfish of the same species, prepared like the samples (Section 7). No positive control (okadaic acid standard) is offered
Storage	All components at room temperature; cassettes in their sealed foil pouches

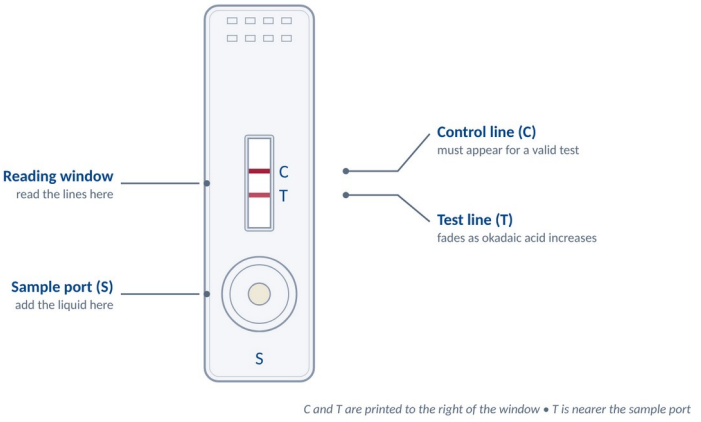


Figure 2. *Parts of the cassette. Add the liquid to the sample port (S) and read the lines through the window. The test line (T) is nearer the sample port than the control line (C).*

In development, oyster and mussel spiked at 160 µg/kg gave test lines clearly weaker than their negative controls: on average, T/C was about 38% lower than the negative control (read with a lateral flow reader).

4. Storage and Stability

- Store the cassettes in their sealed foil pouches at room temperature. Open a pouch only when you are ready to run the test.
- Store the Sample Dilution Buffer, syringes and filters at room temperature.
- Use the kit before the expiration date on the label.

5. Required Materials Not Supplied

Reagents

- **Methanol, 100% (analytical or HPLC grade)** – 4.0 mL per extract
- **2.5 M sodium hydroxide (NaOH)** – dissolve 1.0 g of NaOH pellets in deionized water to 10 mL. 100 µL per extract
- **2.5 M hydrochloric acid (HCl)** – add 2.1 mL of 37% (w/w) HCl slowly to 7.9 mL of deionized water. 100 µL per extract
- **Deionized or distilled water** – to rinse the shellfish and dilute the extracts
- **Okadaic acid-free shellfish** – of the same species as the samples, for the negative control extract (Section 7)

Equipment

- **Shucking knife, sieve and blender** – to prepare the homogenate
- **Balance** – readable to 0.01 g
- **15 mL polypropylene conical tubes with screw caps** – one per extract
- **Glass vials with screw caps (about 4 mL), suitable for heating** – one per extract
- **Heat block or water bath at 76 °C, with a thermometer**
- **Pipettors (20–200 µL and 100–1000 µL) and tips; pipette for 4.0 mL**
- **1.5 mL microcentrifuge tubes, vortex mixer, timer, permanent marker**
- **Lateral flow reader** – recommended, for T/C values (Section 9)
- **Gloves, safety glasses and a lab coat; fume hood** – for work with methanol, NaOH and HCl

6. Precautions

- This kit gives screening results. Confirm positive results, and any result used for a regulatory or harvest decision, by LC-MS/MS or another official method.
- Methanol is toxic and highly flammable. Work in a fume hood, away from flames and sparks, and wear gloves and safety glasses. Follow the supplier's

safety data sheet, and dispose of methanol waste according to local regulations.

- Sodium hydroxide and hydrochloric acid are corrosive. Always add concentrated acid to water, never water to acid.
- The hydrolysis heats methanol above its boiling point (65 °C) in a closed vial. Use glass vials rated for heating, close the caps tightly, heat in a fume hood, and let the vials cool to room temperature before opening them.
- Okadaic acid is a toxin. Treat all shellfish, extracts and used cassettes as contaminated. Wear gloves and wash your hands after testing.
- The Sample Dilution Buffer contains sodium azide as a preservative. Do not swallow it, and rinse any skin or eye contact with water.
- Use new tips, syringes, filters, vials and tubes for each sample. Use only reagents from one kit lot; the components of a lot have been adjusted to work together. Do not use a cassette from a damaged pouch. Use each cassette once.
- Lay each cassette flat on a level surface and do not move it while the sample runs.

7. Sample Collection and Preparation

Collect and hold

- Keep live or whole shellfish at 2–8 °C and test within 48 hours of harvest.
- For longer holding, shuck and homogenize the tissue (below) and freeze the homogenate at −20 °C or colder. Thaw completely and mix well before weighing.

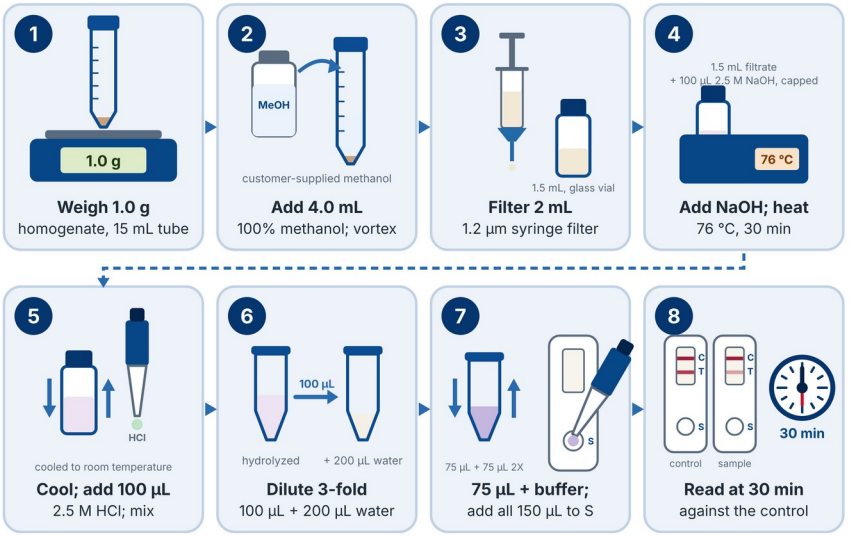
Homogenize

- Rinse the outside of the shellfish with deionized or distilled water to remove sand and mud. Shuck at least 10 to 12 animals, to give at least 100 g of tissue for each sample. Use the whole edible tissue.
- Drain the tissue on a sieve for about 5 minutes, then homogenize it in a blender until it is smooth and uniform. Bring the homogenate to room temperature before weighing.

Negative control extract

- Prepare a homogenate of shellfish known to be free of okadaic acid, of the same species as the samples, in the same way. Extract it alongside the samples (Section 8) for every negative control cassette. Do not use one species as the negative control for another.

8. Procedure



Before you start

- Set the heat block or water bath to 76 °C and check the temperature with a thermometer.
- Bring the cassettes, Sample Dilution Buffer and homogenates to room temperature.
- Each sample needs two cassettes and two Sample Dilution Buffer tubes: one set for the sample extract and one for the negative control extract.

A. Extract (each sample and the negative control homogenate)

1. Weigh 1.0 g (± 0.05 g) of homogenate into a 15 mL polypropylene conical tube.
2. Add 4.0 mL of 100% methanol. Cap the tube tightly and vortex for 30 seconds.
3. Draw 2 mL of the mixture into a syringe, attach a 1.2 µm syringe filter and push the liquid through the filter into a glass vial. About 1.5 mL of filtrate is recovered.

B. Hydrolyze

4. Add 100 µL of 2.5 M NaOH to 1.5 mL of filtrate in the glass vial. Cap the vial tightly and shake to mix.
5. Heat the vial for 30 minutes at 76 °C.
6. Let the vial cool to room temperature, then open it.
7. Add 100 µL of 2.5 M HCl, cap and shake to mix. This is the hydrolyzed extract.

C. Dilute and run the test

8. Transfer 100 µL of hydrolyzed extract into a 1.5 mL tube containing 200 µL of deionized water (3-fold dilution). Mix by pipetting. This is the diluted extract.
9. Wearing gloves, take the cassettes out of their pouches and lay them flat on a level surface. Label each cassette on the plastic (not on the window), for example “NC” and the sample name.
10. Spin the Sample Dilution Buffer tubes briefly so that all the buffer is at the bottom of each tube.
11. Pipette 75 µL of diluted extract into a Sample Dilution Buffer tube (75 µL of 2X buffer). Mix by pipetting up and down 3 times.
12. Transfer the entire contents of the tube (about 150 µL) into the sample port (S) of the cassette, all at once.
13. Repeat steps 11 and 12 with the diluted negative control extract and the negative control cassette.
14. Start the timer as soon as both cassettes are loaded. Leave the cassettes flat and undisturbed for 30 minutes, then read both cassettes (Section 9).

NOTE: read at 30 minutes, unless your own testing shows that a different time is better for your application. Read the sample cassette and its negative control cassette at the same time. Do not add any further liquid to the cassette after the sample. Test the diluted extracts on the day they are made.

9. Interpretation of Results

The test is set to call samples at the regulatory limit of 160 µg/kg. Read the sample cassette and its negative control cassette side by side. First check the control line (C), then compare the test lines (T) (Table 1 and Figure 3).

Result	What you see	Meaning
Invalid	No control line (C) on the sample cassette or on the negative control cassette	The test did not run correctly. Repeat with new cassettes (Section 11).
Negative	Sample T line as strong as, or nearly as strong as, the T line of the negative control	Okadaic acid below about 160 µg/kg
Positive	Sample T line clearly weaker than the T line of the negative control, or absent	Okadaic acid at or above about 160 µg/kg. Confirm by an official method

Table 1. How to read the result by eye. The intensity of the control line varies between cassettes and does not affect the result, as long as a control line is visible.

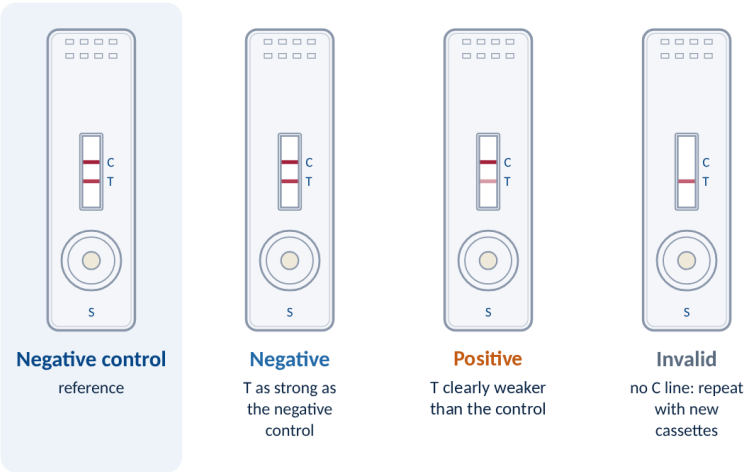


Figure 3. *Reading the result by eye, against the negative control. Negative: test line as strong as the negative control. Positive: test line clearly weaker than the negative control, or absent (about 160 µg/kg or above). Invalid: no control line.*

Reading with a lateral flow reader

Calculate T/C (test line value ÷ control line value) for the sample and for its negative control, and the drop: (negative control T/C – sample T/C) ÷ negative control T/C. A drop of about 38% or more is positive (okadaic acid at or above about 160 µg/kg). The 38% cutoff is the average for oyster and mussel spiked at 160 µg/kg in development. It is an estimate: establish the cutoff formally for your own samples by LC-MS/MS or with a set of spiked standards (see the cutoff control below).

Cutoff control (optional, recommended)

For the most reliable call, run a cutoff control with each batch: spike 1.0 g of the negative control homogenate with okadaic acid standard (not supplied) to 160 µg/kg (160 ng; for example 16 µL of a 10 µg/mL standard), let it stand 30 minutes at room temperature, and extract and test it like the samples. A sample whose T line is as weak as, or weaker than, the cutoff control (T/C at or below the cutoff control T/C) is positive.

10. Limitations

- The test is a screen at 160 µg/kg; it does not give an exact concentration. Confirm positive results by LC-MS/MS or another official method (Section 12).

- Cross-reactivity with the dinophysistoxins DTX1, DTX2 and DTX3 is to be determined.
- Tissue changes the response: results can differ between species and between tissues of different condition or storage history.
- The negative control must be an extract of the same species prepared in the same way. A water-based or methanol-only control does not match the T/C of a tissue extract.
- The procedure has been tested in development with oyster and mussel only; validate it for other species before routine use.

11. Troubleshooting

Observation	Likely cause	What to do
No control line	Too little liquid in the sample port, cassette moved or tilted during the run, or cassette exposed to moisture	Add the full volume to the sample port; keep the cassette flat and still; use a new cassette from an undamaged pouch
No or weak test line on the negative control cassette	Extract not diluted, too much methanol or tissue in the test, or cassette damaged or expired	Check the 3-fold dilution and the volumes (Section 8); check the expiration date; repeat with new cassettes. If it happens again, contact Attogene
Filter hard to push or clogs	Tissue lumps or a heavy particle load	Vortex until no lumps remain, let the solids settle for 1 minute and draw the liquid from the top; use a new filter
Less than 1.5 mL of filtrate	Liquid held in the filter or the solids	Filter a further portion of the mixture with a new filter and combine the filtrates
Results vary between repeats of one sample	Homogenate not uniform, or vial temperature not reached	Homogenize until smooth and mix before weighing; check the heat block with a thermometer
Sample and negative control hard to compare	Small difference in test line, or cassettes read at different times	Read both cassettes at 30 minutes under the same light; use a lateral flow reader or a cutoff control (Section 9)

12. Additional Analysis

If needed, confirm positive samples by LC-MS/MS or another official method for lipophilic shellfish toxins. Contact us with any questions.

13. Ordering Information

Catalog no.	Description	Size
AU2072	Okadaic Acid Lateral Flow Kit, Field Use (fresh and seawater): 10 cassettes, buffer, negative controls, syringes, filters and sample bottles	5 samples
AU2072-01	Okadaic Acid Lateral Flow Kit, Laboratory Use (fresh and seawater): 10 cassettes, buffer and negative controls	5 samples
AU2071	Okadaic Acid Lateral Flow Kit, Laboratory Use (shellfish): 10 cassettes, buffer, syringes and 1.2 µm filters; methanol, NaOH and HCl not included	5 samples

For bulk cassettes, custom formats or other toxins, contact sales@attogene.com.

Contact Us

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Austin, TX 78744
Phone: 512-333-1330
Email: sales@attogene.com
Web: www.attogene.com

14. Quick Reference

Procedure at a glance

Step	Action
1	Weigh 1.0 g of homogenate (sample, and negative control of the same species) into 15 mL tubes. Add 4.0 mL of 100% methanol; vortex.
2	Filter 2 mL through a 1.2 µm syringe filter into a glass vial (about 1.5 mL).
3	Add 100 µL of 2.5 M NaOH; cap; heat 30 minutes at 76 °C; cool to room temperature.
4	Add 100 µL of 2.5 M HCl; mix.
5	Dilute 3-fold: 100 µL of hydrolyzed extract + 200 µL of deionized water.
6	75 µL of diluted extract into a Sample Dilution Buffer tube; mix 3 times; add the entire contents (about 150 µL) to port S.
7	Leave flat; read both cassettes at 30 minutes.

Reading the result (always against the negative control extract)

Result	Sample cassette
Invalid	No C line (on either cassette): repeat
Negative	T line as strong as, or nearly as strong as, the negative control: below about 160 µg/kg
Positive	T line clearly weaker than the negative control, or absent: at or above about 160 µg/kg
Reader	Drop in T/C of about 38% or more, or T/C at or below the cutoff control: positive

Storage

- All kit components at room temperature; open a cassette pouch only when ready to test.
- Shellfish: 2–8 °C for up to 48 hours; homogenate frozen at –20 °C or colder.

Notes

Notes

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