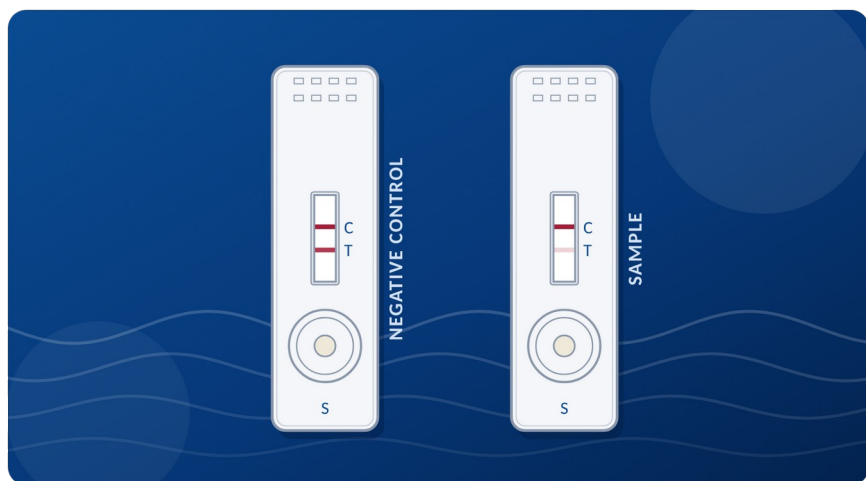




Tetrodotoxin Lateral Flow Test

Tetrodotoxin • Colloidal Gold • Laboratory Use

Catalog Number: **AU2047**

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

1. Introduction

Attogene's Tetrodotoxin Lateral Flow Test is a rapid, single-use cassette test that screens water and tissue extracts for tetrodotoxin (TTX) at a visual screening level of 10 ppb (10 µg/L); with a lateral flow reader it detects to about 1.5 ppb. Each sample is mixed with the supplied running buffer and added to a cassette with the supplied fixed-volume pipette. The kit is for research and screening use and is a convenient basis for developing a TTX lateral flow assay.

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

Important: a weaker test line means MORE TTX. Always read each sample against a negative control cassette run at the same time (Section 9).

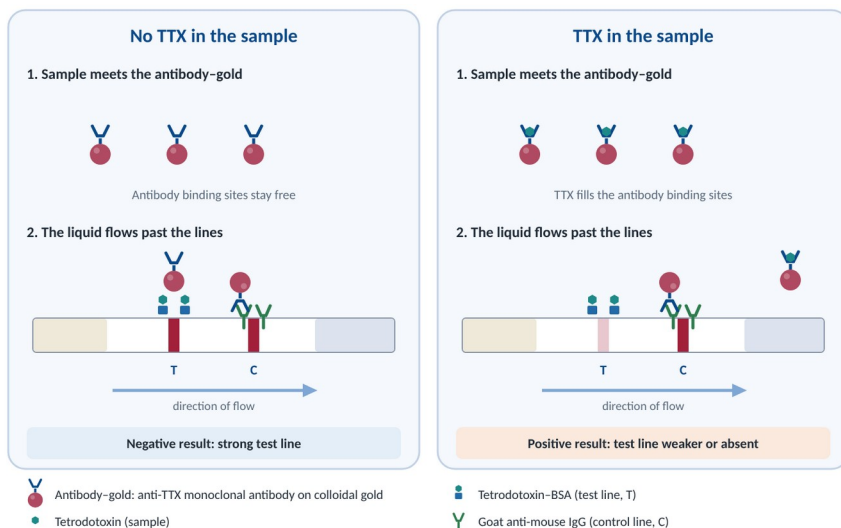


Figure 1. Test principle. Left: with no TTX, the antibody-gold binds the tetrodotoxin-BSA test line (T) and the excess binds the control line (C). Right: TTX in the sample blocks the antibody, so the test line is weaker or absent; the control line still forms.

About tetrodotoxin

Tetrodotoxin (TTX) is a potent, heat-stable neurotoxin (molecular weight about 319 Da) found in pufferfish and some other marine organisms. It blocks voltage-gated sodium channels and can cause paralysis; poisoning most often follows the improper preparation or consumption of pufferfish.

Because TTX is potent and stable, rapid screening of water and tissue extracts is useful in food-safety and environmental monitoring. This kit provides a qualitative screening result; confirm positive results by a conventional analytical method (Section 12).

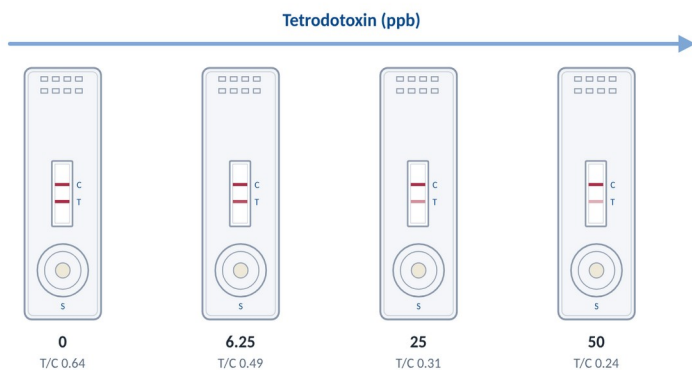


Figure 2. TTX tested on AU2047 cassettes across a 0–50 ppb series (QC, 2025). The cassette images are redrawn from the lateral flow reader values ($T/C = \text{test line value} \div \text{control line value}$): the test line weakens as TTX increases, while the control line stays about the same. The image is for illustration; line intensities vary between lots and with the sample.

Features & Benefits

- Screens water and tissue extracts for TTX at a visual screening level of 10 ppb; a lateral flow reader detects to about 1.5 ppb.
- Results in 15 minutes; no instrument is needed for the visual result. A lateral flow reader can be used for numerical (T/C) readings.
- A competitive format: the test line weakens as TTX increases. Every sample is read against its own negative control cassette.
- A convenient basis for developing and screening a TTX lateral flow assay; 5 samples per kit, each with its own negative control.



2. Kit Contents

Component	Quantity	Storage
TTX Cassette, in a foil pouch with desiccant	10	Room temperature
10X Assay Running Buffer, 15 µL per tube	5 tubes	Room temperature
Negative Control (1X running buffer, no TTX): 150 µL per tube	5 tubes	Room temperature
Manual	1	Room temperature
Sample Dilution Solution (for diluting interfering samples)	5 mL	Room temperature
Fixed-volume pipette, 75 µL	10	Room temperature

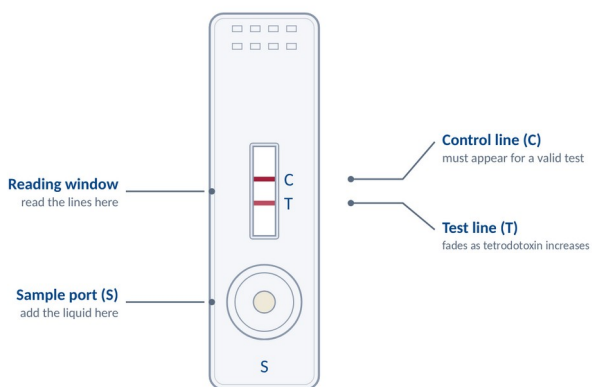
Kit size: 5 samples. Each sample uses two cassettes: one for the sample and one for its negative control. Each cassette is sealed in its own foil pouch with a desiccant. Extra Sample Dilution Solution is available as an add-on (Section 13).



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 3)
Assay principle	Competitive immunoassay: the test line weakens as TTX increases
Reporter	Colloidal gold conjugated to an anti-TTX monoclonal antibody
Test line (T)	Tetrodotoxin-BSA conjugate
Control line (C)	Goat anti-mouse IgG; captures the antibody-gold that passes the test line
Samples	Water; pufferfish tissue; shellfish and clam tissue extracts; gastrointestinal contents; plasma. Prepare tissue samples as a homogenate extract (e.g. 1 g tissue per 30 mL water) and centrifuge before testing.
Screening level	Visual: 10 ppb (10 µg/L); reader: about 1.5 ppb TTX in the sample
Visual detection	Test line visibly weaker than the negative control at about 10 ppb; T/C falls from about 0.64 (0 ppb) to 0.24 (50 ppb) by reader (Figure 2)
Sample preparation	150 µL of sample mixed with the 10X Assay Running Buffer in the tube
Volume to cassette	150 µL (2 × 75 µL with the fixed-volume pipette); 150 µL of Negative Control
Time to result	Read at 15 minutes
Controls	Negative Control supplied, one per sample. Positive control (TTX standard) not supplied
Cross-reactivity	Anti-TTX monoclonal antibody; high specificity for tetrodotoxin. Cross-reactivity with TTX analogs (e.g. 4,9-anhydroTTX, deoxy-TTX) and other marine neurotoxins (e.g. saxitoxin) is under characterization through ongoing research. Contact Attogene for current specificity data or to screen for a specific analog using a verified CRM.
Storage	All components at room temperature; cassettes in their sealed foil pouches



C and T are printed to the right of the window • T is nearer the sample port

Figure 3. 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).

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 Solution and the Negative Control at room temperature.
- Use the kit before the expiration date on the label.

5. Required Materials Not Supplied

- **Timer** – to time the run
- **Disposable gloves** – for sample collection and testing
- **Microcentrifuge** – to clear turbid samples or tissue extracts before testing
- **Clean tubes** – for preparing or diluting samples
- **Clean sample collection bottles** – one per sample
- **Permanent marker** – to label bottles and cassettes

6. Precautions

- This kit gives screening results. Confirm positive results, and any result used for a regulatory decision, by LC-MS/MS, ELISA or another conventional analytical method.

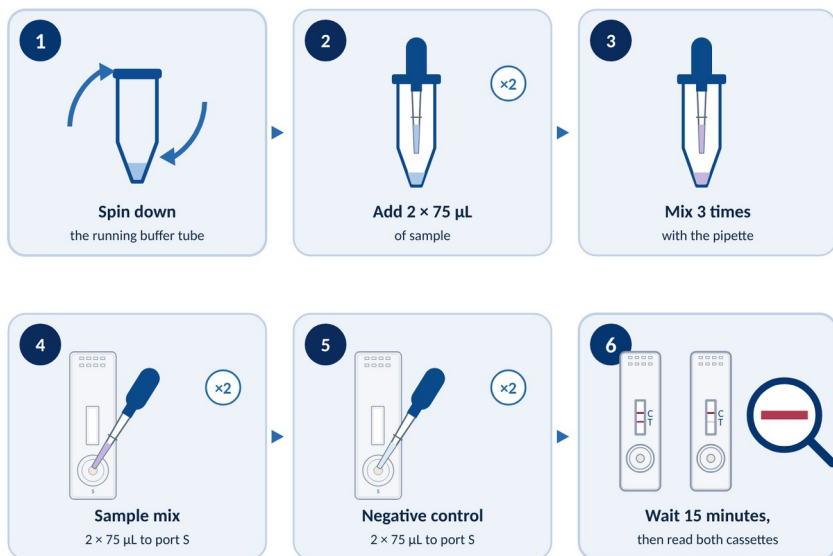


- Before use, check that the date of use is before the expiration date on the label.
- TTX is a potent neurotoxin. Wear gloves when you handle samples, and wash your hands after testing.
- The running buffer and the Negative Control contain sodium azide as a preservative. Do not swallow them, and rinse any skin or eye contact with water.
- Use a new running buffer tube and a new fixed-volume pipette for each sample to avoid cross-contamination.
- 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.
- Good laboratory practice: test a sample of known TTX concentration (positive control, not supplied) with each kit lot to see the line intensity to expect.
- Matrix interference: some samples can interfere with the assay. If interference is suspected, pre-dilute the sample in the Sample Dilution Solution before adding it to the running buffer.

7. Sample Collection and Storage

- Wearing gloves, collect the sample into a clean bottle and label the bottle.
- Test samples as soon as possible. Samples can be stored refrigerated (2–8 °C) for up to 5 days; freeze samples that must be held longer.
- For a tissue sample, prepare an extract (for example 1 g of tissue in 30 mL of water), then centrifuge or filter it so the liquid added to the test is clear. If a water sample is turbid, centrifuge it and test the clear supernatant.
- Bring the cassettes, the Sample Dilution Solution, the Negative Control and the samples to room temperature, and mix the samples, before testing.

8. Procedure



Before you start

- Bring the cassettes, Sample Dilution Solution, Negative Control and samples to room temperature.
- Each sample needs two cassettes: one for the Negative Control and one for the sample.
- 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.
- Tap the 10X Assay Running Buffer tube on the bench so that all the buffer is at the bottom of the tube. If the sample may interfere, pre-dilute it in the Sample Dilution Solution first.

A. Sample

1. With the $75 \mu\text{L}$ fixed-volume pipette, draw up sample to the fill mark and dispense it into a running buffer tube. Repeat once, for $150 \mu\text{L}$ in total.
2. Mix by pipetting up and down 3 times.
3. Mix gently by drawing the liquid into the pipette and releasing it, 3 times. Transfer $150 \mu\text{L}$ ($2 \times 75 \mu\text{L}$) of the mixed sample into the sample port (S) of the sample cassette.



B. Negative control

4. With a new fixed-volume pipette, add 150 μL ($2 \times 75 \mu\text{L}$) of Negative Control into the sample port (S) of the negative control cassette.

C. Run and read

5. Start the timer as soon as both cassettes are loaded. Leave the cassettes flat and undisturbed for 15 minutes.
6. Read both cassettes by eye or with a lateral flow reader (Section 9).

NOTE: read at 15 minutes, with the sample cassette and its negative control cassette side by side.

9. Interpretation of Results

Read the sample cassette and its negative control cassette side by side. First check the control line (C), then compare the test line (T) of the sample with the test line of the negative control (Table 1 and Figure 4).

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 stronger than, the T line of the negative control	No TTX detected (below about 10 ppb)
Low positive	Sample T line clearly weaker than the negative control but visible	TTX detected, about 10 ppb and above
Positive	Sample T line much weaker or absent	High TTX

Table 1. How to read the result. The control line stays about the same between cassettes; a visible control line means the result is valid.

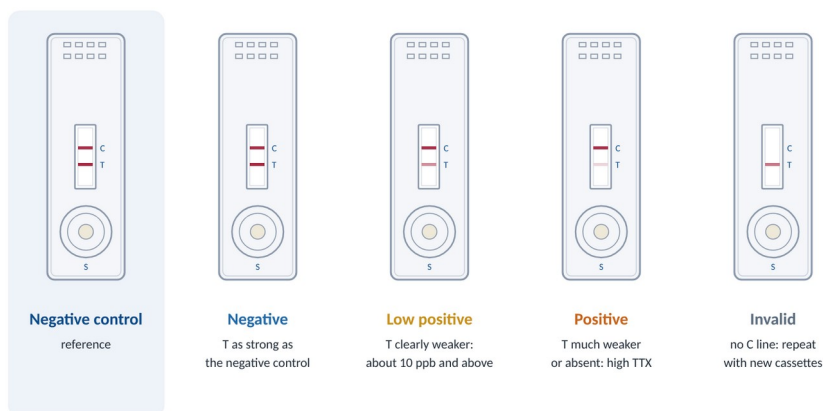


Figure 4. Reading the result against the negative control. *Negative: test line as strong as the negative control. Low positive: test line clearly weaker (about 10 ppb and above). Positive: test line much weaker or absent (high TTX). Invalid: no control line.*

Reading with a lateral flow reader

Calculate T/C (test line value $\div$ control line value) for the sample and for its negative control, and compare the two. In kit lot testing (Figure 2), T/C was about 0.64 at 0 ppb, 0.49 at 6.25 ppb, 0.31 at 25 ppb and 0.24 at 50 ppb; a lower T/C than the negative control means more TTX.

Approximate concentrations (semi-quantitative)

To estimate concentration, test TTX standards of known concentration (not supplied), prepared in the same type of sample as the test sample, alongside the samples, and compare the test lines. Do not use cassettes run earlier.

10. Limitations

- The test screens for TTX; it does not give an exact concentration. Confirm positive results by a conventional analytical method (Section 12).
- Some sample matrices can interfere with the assay. If interference is suspected, pre-dilute the sample in the Sample Dilution Solution and retest (Section 8).
- The test is characterized against TTX; its response to other sodium-channel toxins has not been established. To characterize the response to a particular toxin or sample matrix, create a standard curve with a verified certified reference material (CRM) of that material.
- Very turbid samples or tissue extracts can slow the flow. Centrifuge or filter such samples before testing (Section 7).



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 150 μ L ($2 \times 75 \mu$ L) to the sample port; keep the cassette flat and still; use a new cassette from an undamaged pouch
No test line on the negative control cassette	Cassette damaged or expired, or the Negative Control was not added	Check the expiration date; repeat with new cassettes. If it happens again, contact Attogene
Slow flow, liquid left in the port, or high background	Particles or algae in the sample	Centrifuge or filter the sample and use the clear liquid (Section 7)
Sample and negative control hard to compare	Cassettes read at different times or under different light	Read both cassettes side by side, under the same light, at 15 minutes
Sample test line weak even at low TTX	Matrix interference	Pre-dilute the sample in the Sample Dilution Solution and retest

12. Additional Analysis

If needed, confirm positive samples by LC-MS/MS, ELISA or another conventional analytical method. A lateral flow reader can be used to obtain numerical (T/C) readings from the visual result. Contact us with any questions.

13. Ordering Information

Catalog no.	Description	Size
AU2047	Tetrodotoxin Lateral Flow Test, Laboratory Use (colloidal gold): 10 cassettes, 10X Assay Running Buffer, negative controls, Sample Dilution Solution and fixed-volume pipettes	5 samples

Add-ons

Extra Sample Dilution Solution can be bought separately as an add-on, in a 5-pack or a 10-pack, from www.attogene.com.

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



Contact Us

3913 Todd Lane, Suite 310

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	Bring everything to room temperature. Lay two cassettes flat per sample: "NC" and sample.
2	Tap down the 10X running buffer tube. Add $2 \times 75 \mu\text{L}$ of sample with the fixed-volume pipette.
3	Mix 3 times. Transfer $2 \times 75 \mu\text{L}$ of the mixed sample to port S of the sample cassette.
4	Add $2 \times 75 \mu\text{L}$ of Negative Control to port S of the negative control cassette.
5	Start the timer; leave both cassettes flat and undisturbed.
6	Read both cassettes at 15 minutes.

Reading the result (always against the negative control)

Result	Sample cassette
Invalid	No C line (on either cassette): repeat
Negative	T line as strong as or stronger than the negative control: no TTX detected
Low positive	T line clearly weaker than the negative control: TTX about 10 ppb and above
Positive	T line much weaker or absent: high TTX

Storage

- All components at room temperature; open a cassette pouch only when ready to test.
- Use the kit before the expiration date on the label.







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