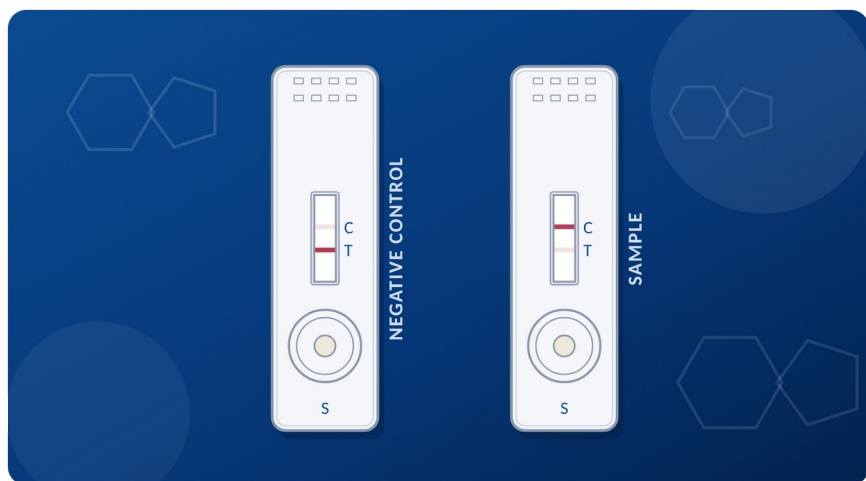




Caffeine Lateral Flow Kit

Urine, Water and Drinks • Laboratory Use

Catalog Number: **AU2067**

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



1. Introduction

Attogene's Caffeine Lateral Flow Kit is a rapid, single-use cassette test that screens liquids for caffeine in 15 minutes. The cutoff is 10 ppb (0.01 ppm) caffeine in the Sample Dilution/Running Buffer tube (final concentration): at 10 ppb and above, the control line (C) is clearly darker than on the negative control, and on a lateral flow reader the T/C ratio falls to less than half that of the negative control. This version is made for the laboratory and is compatible with urine, water and drinks. 1 to 20 μL of sample is added to a 200 μL tube of Sample Dilution/Running Buffer, mixed and added to a cassette. The sample volume sets the concentration in the sample that corresponds to the cutoff (Section 7, Table 1): with 20 μL of urine or water it is about 110 ppb, and with 1 μL of a drink about 2 ppm.

The test is a competitive immunoassay (Figure 1). As the sample flows along the strip, it picks up colloidal gold coated with an anti-caffeine antibody. The test line (T) carries caffeine bound to BSA. If the sample contains no caffeine, the gold binds the test line and a strong red test line forms. Caffeine in the sample fills the antibody binding sites, so less gold binds the test line and more gold travels on to the control line (C), goat anti-mouse IgG. The more caffeine in the sample, the weaker the test line and the darker the control line.

Important: in this kit the control line is faint, and may not be visible at all, when the sample contains no caffeine. This is normal. Caffeine makes the test line weaker and the control line darker. Always read each sample against a negative control cassette run at the same time (Section 9).

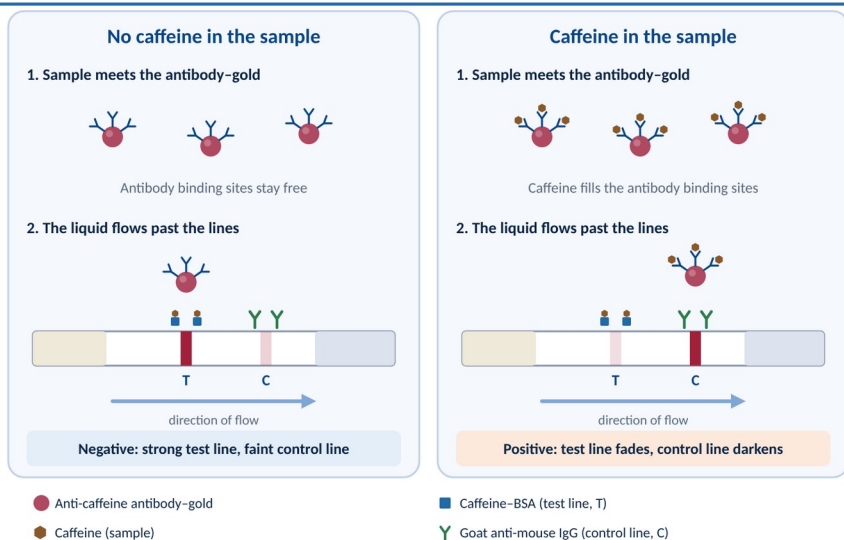


Figure 1. Test principle. Left: with no caffeine, the antibody-gold binds the caffeine-BSA test line (T); little gold reaches the control line (C). Right: caffeine in the sample blocks the antibody, so the test line is weaker or absent and the control line is darker.

About caffeine

Caffeine (1,3,7-trimethylxanthine) is a natural stimulant found in coffee beans, tea leaves, cacao and kola nuts, and is added to colas and energy drinks. Decaffeination removes most, but not all, of the caffeine from coffee. Caffeine is absorbed after drinking and a small part is excreted unchanged in urine. The concentrations in this manual are given in parts per million (ppm, mg/L) and parts per billion (ppb, $\mu\text{g/L}$): 1 ppm = 1,000 ppb.

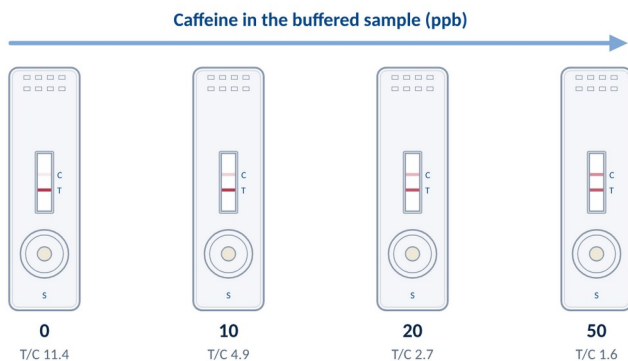


Figure 2. Buffer spiked with caffeine and tested with an AU2067 kit lot (kit QC, lot 202509105, 9/24/2025; read with a lateral flow reader at 15 minutes). The cassette images are redrawn from the reader values ($T/C = \text{test line value} \div \text{control line value}$). The control line darkens from 10 ppb on, and T/C drops from 11.4 at 0 ppb to 4.9 at 10 ppb and 1.6 at 50 ppb. The image is for illustration only and does not represent the performance of any particular lot.

Features & Benefits

- Results in 15 minutes, by eye or with a lateral flow reader.
- Every sample is read against its own negative control cassette.
- Compatible with urine, water and drinks; the sample volume (1–20 μL) sets the detection range.
- Simple pipetting procedure; 5 samples per kit, each with its own negative control.

2. Kit Contents

Component	Quantity	Storage
Caffeine Cassette, in a foil pouch with desiccant	10	Room temperature
Sample Dilution/Running Buffer: 200 μL per tube. Also used as the negative control	10 tubes	Room temperature
Fixed-volume pipette, 200 μL	10	Room temperature
10X Sample Dilution Buffer, for diluting concentrated samples: 10 mL	1 bottle	Room temperature
Manual	1	Room temperature

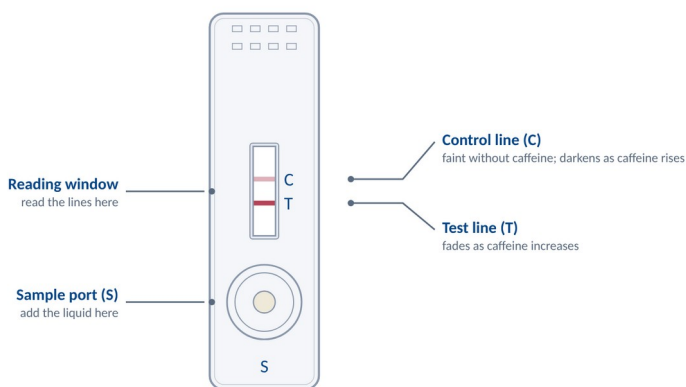


Kit size: 5 samples. Each sample uses two cassettes: one for the sample and one for its negative control. Each cassette is sealed in its own foil pouch with a desiccant.

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, and the control line darkens, as caffeine increases
Reporter	40 nm colloidal gold conjugated to a mouse monoclonal anti-caffeine antibody
Test line (T)	Caffeine-BSA conjugate
Control line (C)	Goat anti-mouse IgG; captures gold that passes the test line. Faint or absent without caffeine
Samples	Urine, water, coffee, tea, soft drinks, energy drinks and other drinks
Cutoff	10 ppb (0.01 ppm) caffeine, final concentration in the Sample Dilution/Running Buffer tube. In the sample: about 110 ppb with 20 μ L of sample, about 2 ppm with 1 μ L (Table 1)
Visual reading	From 10 ppb: C clearly darker than on the negative control (low positive). From about 100 ppb: C as dark as or darker than T, or no T (positive)
Reader reading	From 10 ppb: sample T/C less than half the negative control T/C (kit lot: T/C 11.4 at 0 ppb, 4.9 at 10 ppb; Figure 2)
Sample preparation	1–20 μ L of sample into one 200 μ L tube of Sample Dilution/Running Buffer; mix 3 times
Volume to cassette	200 μ L of the buffered sample; 200 μ L of Sample Dilution/Running Buffer as the negative control
Time to result	Read at 15 minutes, and no later than 20 minutes
Controls	Negative control, one per sample. No positive control (caffeine standard) is supplied
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/Running Buffer tubes and the 10X Sample Dilution Buffer at room temperature. Keep them capped.
- Use the kit before the expiration date on the label.

5. Required Materials Not Supplied

- **Timer** – to time the run
- **Permanent marker** – to label tubes and cassettes
- **Disposable gloves** – for urine samples
- **Pipettor and tips, 1–20 μL** – to add the sample to the buffer tube
- **Clean tubes and a 20–200 μL pipettor** – only to dilute concentrated samples (Section 7)
- **Ultrapure or distilled water** – only to make 1X Sample Dilution Buffer from the 10X Sample Dilution Buffer (Section 7)
- **Clean sample cups** – for urine and water samples
- **Caffeine standard of known concentration (optional)** – as a positive control or to estimate concentration (Section 9)



6. Precautions

- For research use only. Not for use in diagnostic procedures. This kit gives screening results; confirm results used for any decision by HPLC, LC-MS/MS or another conventional analytical method.
- Before use, check that the date of use is before the expiration date on the label.
- The buffers contain sodium azide as a preservative. Do not swallow them, and rinse any skin or eye contact with water. Do not drink any sample after it has been tested.
- Handle urine as potentially infectious. Wear gloves, and dispose of samples and used cassettes as your laboratory requires.
- Use a new pipette for each sample and for each negative control to avoid carrying caffeine from one sample to the next. Coffee contains far more caffeine than the test needs to give a positive result.
- 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 Preparation

Drinks

- Let hot drinks cool to room temperature. Stir carbonated drinks until they stop fizzing. Mix the drink well before sampling.
- To dilute a concentrated sample, first make 1X Sample Dilution Buffer: mix 1 part 10X Sample Dilution Buffer with 9 parts ultrapure or distilled water (for example, 100 μL with 900 μL in a clean tube). Use this 1X buffer for the dilutions below.
- Test 1 μL of each drink (Table 1). To estimate a higher concentration, dilute the drink in 1X Sample Dilution Buffer (for example 1:10: 10 μL of drink into 90 μL), test 1 μL of the dilution and multiply the result by the dilution factor.

Urine and water

- Collect urine or water in a clean cup and test 20 μL of it as it is (Table 1). Mix before sampling.
- Caffeine-free urine reads like the negative control. Caffeine in urine varies with intake and time; to estimate a higher level, dilute the urine 1:10 or more in 1X

Sample Dilution Buffer, test 20 µL of the dilution and multiply the result by the dilution factor.

- If the sample is cloudy, let particles settle for a few minutes and take the sample from the clear top layer, or centrifuge it (2 minutes at 10,000 × g or more) and use the supernatant.
- Test the sample on the day it is collected. If it cannot be tested at once, keep it at 2–8 °C for up to 24 hours, or freeze it at –20 °C or colder. Bring it to room temperature and mix before testing.

Sample volume and cutoff

The cutoff is 10 ppb caffeine in the Sample Dilution/Running Buffer tube after the sample is added. The concentration in the sample that this corresponds to depends on the sample volume (Table 1).

Sample volume (into 200 µL of buffer)	Dilution	Cutoff (10 ppb in the tube), in the sample	Full positive (about 100 ppb in the tube), in the sample
20 µL (urine, water)	1:11	About 110 ppb	About 1.1 ppm
10 µL	1:21	About 210 ppb	About 2.1 ppm
5 µL	1:41	About 410 ppb	About 4.1 ppm
2 µL	1:101	About 1.0 ppm	About 10 ppm
1 µL (drinks)	1:201	About 2.0 ppm	About 20 ppm

Table 1. Concentration in the sample that corresponds to the cutoff and to a full positive, for each sample volume. Use 20 µL for urine and water and 1 µL for drinks.



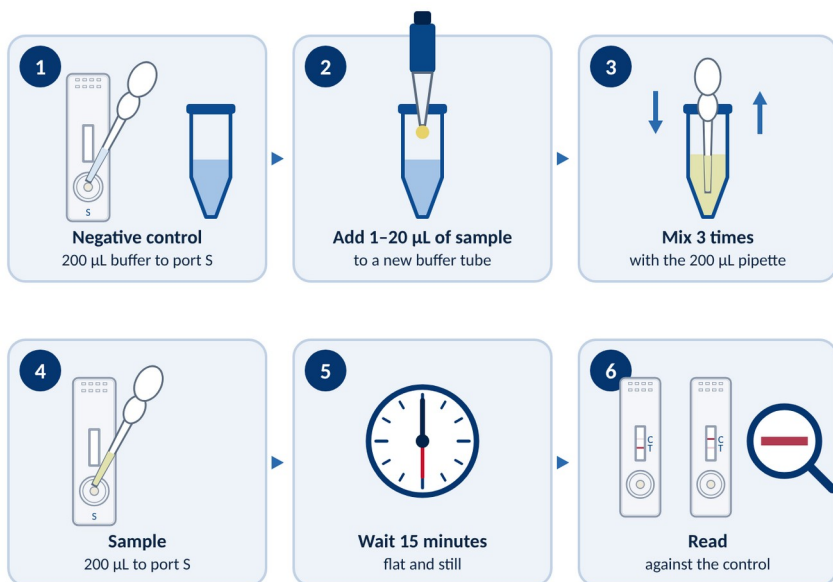
Typical caffeine in drinks

With 1 μL of drink, the 10 ppb cutoff corresponds to about 2 ppm (2 mg/L) in the drink, and a full positive (C as dark as or darker than T) to about 20 ppm or more. Typical values are shown in Table 2; real drinks vary widely.

Drink (8 fl oz, 237 mL unless stated)	Caffeine, mg	About ppm	Expected result
Brewed coffee	96	400	Positive
Espresso (1 fl oz, 30 mL)	63	2,100	Positive
Energy drink	79	330	Positive
Black tea, brewed	48	200	Positive
Cola	33	140	Positive
Green tea, brewed	29	120	Positive
Decaffeinated coffee, brewed	1 (up to about 15)	4 (up to 60)	Low positive (some negative or positive)
Most citrus sodas and root beers	0	0	Negative

Table 2. Typical caffeine content (Mayo Clinic, Caffeine content for coffee, tea, soda and more). ppm values are calculated from the serving size and rounded. Decaffeinated coffees vary; some contain up to about 15 mg per cup.

8. Procedure



Before you start

- Bring the cassettes, buffers and samples to room temperature.
- Each sample needs two cassettes: one for the negative control and one for the sample.
- 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.

A. Negative control (run first)

1. Open a Sample Dilution/Running Buffer tube. With a new 200 μ L pipette, draw up buffer to the fill mark and dispense it into the sample port (S) of the negative control cassette, all at once. Start the timer.

B. Sample

2. Open a new Sample Dilution/Running Buffer tube. With a pipettor, add the sample to the tube: 20 μ L of urine or water, or 1 μ L of a drink (Table 1).
3. With a new 200 μ L pipette, mix by drawing the liquid up and dispensing it back 3 times.
4. Draw up the mix to the fill mark and dispense it into the sample port (S) of the sample cassette, all at once. Note the time.



5. Leave both cassettes flat and undisturbed. Read each cassette 15 minutes after its liquid was added.

NOTE: read at 15 minutes, with the sample cassette and its negative control cassette side by side. Do not add any further liquid to the cassette after the sample.

9. Interpretation of Results

Read the sample cassette and its negative control cassette side by side. First check that the negative control shows a strong test line (T). Then compare the two lines on the sample cassette, and its control line (C) with the control line of the negative control (Table 3 and Figure 4).

Result	What you see	Meaning
Invalid	No line at all on a cassette, or no strong T line on the negative control cassette	The test did not run correctly. Repeat with new cassettes (Section 11).
Negative	Looks like the negative control: strong T line; C line faint or absent, and no darker than on the negative control	No caffeine detected: below the cutoff (10 ppb in the tube)
Low positive	T line still darker than the C line, but the C line clearly darker than on the negative control	Caffeine detected at or above the cutoff (10 ppb in the tube) but below about 100 ppb in the tube; Table 1 gives the concentration in the sample
Positive	C line as dark as or darker than the T line, or no T line	Caffeine detected, about 100 ppb or more in the tube (about 1 ppm or more in 20 μ L of urine or water; about 20 ppm or more in 1 μ L of a drink)

Table 3. How to read the result by eye. Unlike most lateral flow tests, a faint or missing control line is normal for a negative sample in this kit; a run is valid as long as the negative control shows a strong test line and each cassette shows at least one line.

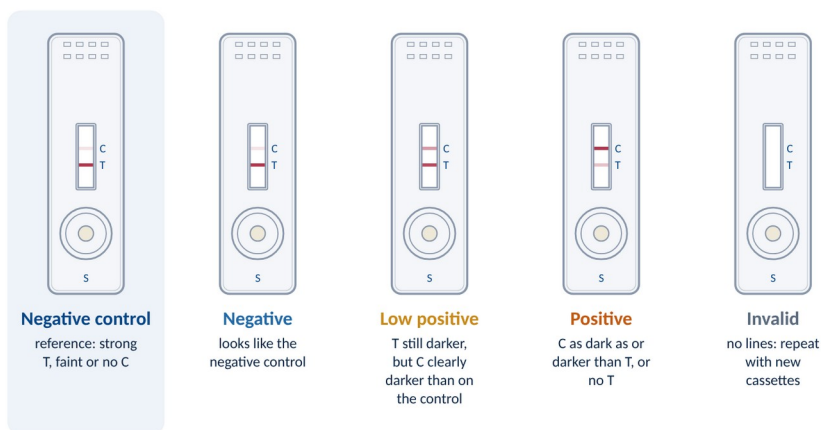


Figure 4. Reading the result by eye, against the negative control. *Negative:* like the negative control. *Low positive:* T still darker than C, but C clearly darker than on the negative control. *Positive:* C as dark as or darker than T, or no T line. *Invalid:* no lines.

Reading with a lateral flow reader (cutoff 10 ppb)

Calculate T/C (test line value ÷ control line value) for the sample and for its negative control, and compare the two. A sample T/C less than half the T/C of its negative control shows caffeine at or above the cutoff. In kit lot testing (Figure 2), T/C was 11.4 at 0 ppb, 4.9 at 10 ppb, 2.7 at 20 ppb and 1.6 at 50 ppb caffeine in the buffered sample.

Approximate concentrations (semi-quantitative)

To estimate concentration, test caffeine standards of known concentration (not supplied), prepared in the same type of liquid as the sample, alongside the samples, and compare the lines. Do not use cassettes run earlier. To estimate a higher concentration, dilute the sample further in 1X Sample Dilution Buffer (Section 7), test it again and multiply the result by the total dilution factor.

10. Limitations

- The test screens for caffeine; it does not give an exact concentration. Confirm results by a conventional analytical method (Section 12).
- Cross-reactivity with related compounds has not been evaluated. These include theobromine (cocoa and chocolate), theophylline (tea) and paraxanthine (the main breakdown product of caffeine in the body, found in urine). They may contribute to a positive result.

- The salt content of the sample affects the control line: salty samples can make it darker and some urines make it fainter. Compare each sample with the negative control and read low positives with care, or use a lateral flow reader.
- Caffeine in urine depends on how much caffeine was taken and when. A negative result does not show that no caffeine was consumed. This kit is not for diagnostic or forensic use.
- Drinks with milk, cream, syrups or pulp have not been evaluated.
- Near the cutoff the control line change is small. Read low positives side by side with the negative control under the same light, or use a lateral flow reader. Concentrations below 10 ppb in the tube (about 110 ppb in 20 μ L of sample) are not reliably detected.

11. Troubleshooting

Observation	Likely cause	What to do
No line at all	Too little liquid in the sample port, cassette moved or tilted during the run, or cassette exposed to moisture	Add 200 μ L to the sample port; keep the cassette flat and still; use a new cassette from an undamaged pouch
Weak or no T line on the negative control	Caffeine carried over from a sample, cassette damaged or expired	Use a new pipette for the negative control; check the expiration date; repeat with new cassettes. If it happens again, contact Attogene
Faint or no C line on a negative sample	Normal for this kit when the sample contains no caffeine	No action needed if the T line is strong
Every drink reads positive, including caffeine-free ones	More than 1 μ L of drink added	Add 1 μ L of drink, or dilute it in 1X Sample Dilution Buffer first (Section 7); decaffeinated coffees usually contain more than 2 ppm and read low positive or positive
Slow flow or liquid left in the port	Particles or pulp in the sample	Let particles settle and test the clear top layer, or centrifuge the sample
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, or use a lateral flow reader



12. Additional Analysis

If needed, confirm results by HPLC, LC-MS/MS or another conventional analytical method. A lateral flow reader can be used to obtain numerical (T/C) readings at the 10 ppb cutoff. Contact us with any questions.

13. Ordering Information

Catalog no.	Description	Size
AU2067	Caffeine Lateral Flow Kit, Laboratory Use (urine, water and drinks): 10 cassettes, Sample Dilution/Running Buffer tubes, 10X Sample Dilution Buffer and fixed-volume pipettes	5 samples
AU2067-01	Caffeine Lateral Flow Kit, Field Use (drinks): 10 cassettes, Sample Dilution Buffer bottles, 10X Running Buffer concentrate and fixed-volume pipettes	5 samples

Extra Sample Dilution Buffer is available in packs of 5 or 10. For bulk cassettes or custom formats, 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	Negative control: 200 µL of Sample Dilution/Running Buffer from a tube into port S. Start the timer.
3	Add the sample to a new buffer tube: 20 µL of urine or water, or 1 µL of a drink. Mix 3 times with a new 200 µL pipette.
4	Add 200 µL of the mix to port S of the sample cassette.
5	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 line at all, or no strong T line on the negative control: repeat
Negative	Like the negative control: strong T, faint or no C
Low positive	T still darker than C, but C clearly darker than on the negative control: 10 ppb cutoff to about 100 ppb in the tube
Positive	C as dark as or darker than T, or no T: about 100 ppb or more in the tube
Reader	Sample T/C less than half the negative control T/C: caffeine at or above the 10 ppb cutoff

Storage

- All components at room temperature; open a cassette pouch only when ready to test.





Notes





Notes



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