



Aquagenx[®] CBT EC+TC Presence/Absence (P/A) Kit Instructions for Use: Drinking Water

Overview

The Aquagenx CBT EC+TC P/A Kit simultaneously detects *E. coli* (EC) and Total Coliform (TC) bacteria in a 100 mL sample. It uses a proprietary powder growth medium with a glucose substrate called X-Gluc. When *E. coli* metabolize this substrate in Aquagenx's growth medium, the color of the water turns blue, indicating the presence of *E. coli*. The growth medium also contains a fluorogenic galactoside substrate called MUGal. If total coliforms are present, they metabolize this fluorogenic substrate and the sample fluoresces blue under a UV light (365 nm). The total coliform group of bacteria includes *E. coli*, which is a fecal coliform as well as a thermotolerant coliform. Not all total coliforms are *E. coli*.

Instructions for testing surface and waste waters: <https://www.aquagenx.com/dilutions-cbt-ectc/>

How-to-use videos and product documents: <https://www.aquagenx.com/product-documentation/>

Shelf Life of Growth Medium

Aquagenx EC+TC powder growth medium is stable up to two-years after date of manufacture at 25° Celsius. Expiration date and lot number are printed on the medium packet.

Storage of Growth Medium

Recommended storage temperature is 10-25° Celsius. Growth medium can be stored in a refrigerator. Cold chain for Aquagenx EC+TC growth medium is not required.

Summary of Test Procedures for CBT EC+TC P/A Kit



How to Interpret Color-Change Test Results

Color in Thio-Bag	Yellow/Yellow Brown in ambient light and does not fluoresce blue under UV light	Yellow/Yellow Brown that ... fluoresces blue under UV light	Blue/Blue Green in ambient light	Blue/Blue Green that ... fluoresces blue under UV light
	<i>E. coli</i>	Negative	Negative	Positive
Total Coliforms	Negative	Positive	Positive	Positive

PROCEDURAL NOTES

1. Sanitize work area with disinfectant cleaning solution, paper towels or wipes.

2. Collect 100 mL water sample with Whirl-Pak® Thio-Bag®

- Wearing disposable, thin plastic gloves is recommended. If you don't have gloves, do not touch inside of Thio-Bag with bare hands.
- Label Thio-Bag or attach barcode asset tag to Thio-Bag
- White tablet in Thio-Bag is sodium thiosulfate, which neutralizes residual chlorine if present in sample. Do not remove.
- Fill Thio-Bag to 100 mL fill mark. Record sample details.

3. Add Aquagenx EC+TC growth medium to sample in Whirl-Pak Thio-Bag

- We recommend testing procedure begins within six hours of sample collection. Do not add growth medium to the Thio-Bag until you are ready to complete the entire testing procedure.
- Open growth medium packet with scissors and pour powder growth medium into Thio-Bag.
- Do not touch growth medium with bare fingers or hands.
- Roll down Whirl-Pak seal and close Thio-Bag shut.
- Dissolve medium in sample. Gently swirl the bag and squeeze clumps of powder until medium is dissolved.

4. Incubation Period and Temperatures

- During the incubation period, CBTs can develop an odor. To control odor, place CBTs in another sealed plastic bag or container during the incubation period.
- Ambient temperature incubation works at any temperatures between 25°- 44.5°C for detection of *E. coli* and/or total coliforms.
- Because the CBT works at variable temperatures, constant temperature control in an incubator is not required. However, at cooler temperatures, constant temperature incubation is recommended, if available.
- Note: over 40°C, some total coliforms will be inhibited, and the results may not be accurate for total coliform analysis.
- For regulatory compliance purposes, samples must be incubated at 35-37°C for 20-24 hours to detect *E. coli* and total coliforms.
- The CBT also can be used to detect thermotolerant (fecal) coliforms instead of total coliforms if the CBT samples are incubated at a temperature of 44.5°C (between 44-45°C) throughout an incubation period of 20-24 hours. Strict temperature control is required for this procedure.

Recommended Incubation Periods at Ambient Temperature Conditions:

35-37°C: Incubate 20 hours

31-34°C: Incubate 24-30 hours

25-30°C: Incubate 40-48 hours

Below 25°C: Incubate in a portable incubator at 35-37°C for 24 hours or put in or near another heat source for up to 48 hours, depending on the temperature.

Over 40°C: Some total coliforms will be inhibited, and the results may not be accurate for total coliforms.

Additional "Incubation Period Guidance": <https://www.aquagenx.com/product-documentation/>

5. View color in Thio-Bag to determine P/A test results (also see color chart on page 1)

E. coli – view in ambient light:

- Yellow/yellow-brown is negative for *E. coli* (absence).
- Blue/blue-green is positive for *E. coli* (presence). Positive results include any trace of blue/blue-green, such as one or more specks of blue/blue-green, or blue/blue-green sediment at bottom of Thio-Bag.

Total Coliform - shine UV light (365 nm) on Thio-Bag in dark environment:

- Samples that fluoresce blue are positive for total coliforms. These include samples that are yellow/yellow-brown in ambient light that fluoresce blue under UV light.
- Samples that are blue/blue-green in ambient light (positive for *E. coli*) are by definition also positive for total coliforms.
- Record test results.

6. Decontaminate sample

- Add 4 mL of liquid bleach (NaOCl) or sufficient chlorine tablets (calcium hypochlorite or sodium dichloroisocyanurate) to Thio-Bag to provide about 200 milligrams of free chlorine.
- After 30 minutes, pour contents into a sink, toilet or hole in ground and safely dispose the empty Thio-Bag.