



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/>

Product documents: <https://www.aquagenx.com/product-documentation/>

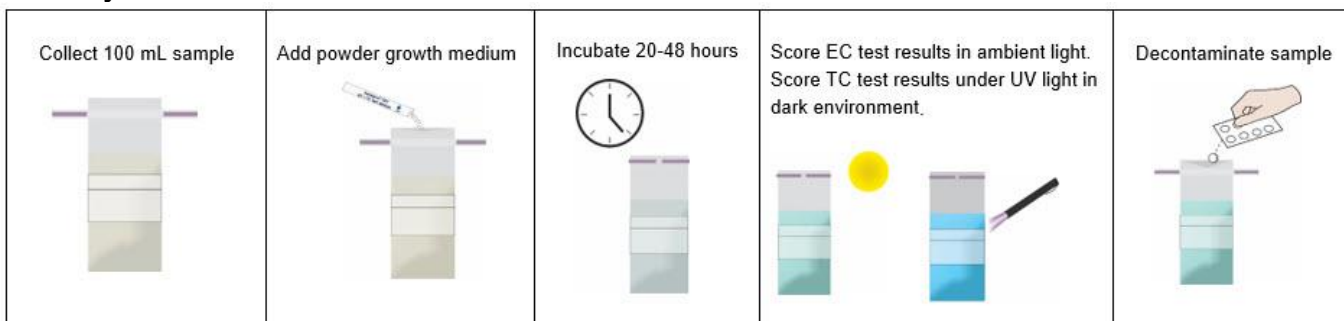
Shelf Life of Growth Medium

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 ...	Blue/Blue Green in ambient light	Blue/Blue Green that...
		fluoresces blue under UV light		fluoresces blue under UV light
<i>E. coli</i>	Negative	Negative	Positive	Positive
Total Coliforms	Negative	Positive	Positive	Positive

PROCEDURAL NOTES. SEE HOW-TO VIDEOS: <https://www.aquagenx.com/how-to-use-cbt-ectc/>

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 it from the bag.
- Fill Thio-Bag to 100 mL fill mark. Record sample details such as date, time and location.

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 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.

See "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.