



Aquagenx Compartment Bag Test (CBT)

CBT *E. coli* Kit Instructions for Use: 1:10 Dilution Recreational Waters, Surface Waters

Overview

The Aquagenx CBT *E. coli* Kit detects and quantifies the Most Probable Number (MPN) of *E. coli* in a 100 mL sample. It uses a proprietary chromogenic growth medium with a glucose substrate called X-Gluc. When *E. coli* metabolize Aquagenx's growth medium, the color of the water turns blue, indicating the presence of *E. coli*. The MPN level of *E. coli* in the sample is estimated by the combination of positive and negative compartments in the Aquagenx compartment bag. Test results are obtained by easy color match using the Aquagenx color-coded MPN Table.

Shelf Life

Aquagenx *E. coli* growth medium (test bud) is stable up to 2-years after date of manufacture at 25-30° Celsius

Storage

Cold chain for *E. coli* growth medium not required. Recommended storage temperature for test bud is 15-25° Celsius. It is also safe to store test buds in a refrigerator. Protect *E. coli* growth medium from bright light.

How to Use Aquagenx CBT *E. coli* Kit for 1:10 Dilution

Collect sample	Add test bud	Pour sample into compartment bag	Attach plastic clip and roll down Whirl-Pak seal

Incubate 24 - 48 hours	Score MPN test results	Decontaminate sample



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Aquagenx CBT Most Probable Number (MPN) Table 1:10 Dilution

The Aquagenx MPN Table is adapted for measuring *E. coli* in a 1:10 dilution with the Compartment Bag Test. Risk categories are based on U.S. EPA Recreational Water Criteria Recommendation 1 values and risk values for *E. coli* in fresh water.

Align your compartment bag so compartment #1 is on the left and compartment #5 is on the right. Match the color sequence of your five compartments to one of these 32 rows:



Compartment Number					MPN/100mL	Upper 95% Confidence Level/100mL	U.S. EPA Recreational Water Health Risk Category Based on MPN and Upper 95% Confidence Level
1	2	3	4	5			
10mL	30mL	56mL	3mL	1mL			
					0	28.70	Low Risk/Safe
					10	51.40	Low Risk/ Probably Safe
					10	47.40	
					11	51.60	
					12	56.40	
					15	78.10	
					20	63.20	
					21	68.50	
					21	66.40	
					24	78.10	
					24	81.20	
					26	85.10	
					32	83.80	
					37	97.00	
					31	113.60	
					32	118.20	
					34	125.30	
					39	104.30	
					40	109.40	
					47	227.50	Low Risk/Possibly Safe
					52	147.30	
					54	129.30	
					56	171.40	
					58	168.70	
					84	211.90	Intermediate Risk/Possibly Safe
					91	370.40	
					96	376.80	High Risk/Probably Unsafe
					136	830.60	
					171	563.50	Very High Risk/Unsafe
					326	1455.50	
					483	3519.10	Very Unsafe
					>1000	94351.00	

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U.S. EPA 2012 Recreational Water Quality Criteria

Note: Aquagenx MPN Table risk categories are based on EPA Recommendation 1 for *E. coli*

CRITERIA ELEMENTS	Recommendation 1 Estimated Illness Rate 36/1,000		Recommendation 2 Estimated Illness Rate 32/1,000	
	GM (cfu/100 mL)	STV (cfu/100 mL)	GM (cfu/100 mL)	STV (cfu/100 mL)
Enterococci (marine and fresh)	35	130	30	110
<i>E. coli</i> (fresh)	126	410	100	320

Source: U.S. EPA Office of Water, EPA-820-F-12-061, Recreational Water Quality Criteria Fact Sheet

Procedural Notes

A short video on how to use the CBT *E. coli* Kit is on the Aquagenx website:

<https://www.aquagenx.com/how-to-use-the-cbt/>

1. Prepare work area

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

2. Collect 10 mL water sample

- White particles in sample bottle and white tablet in Thio Bag are sodium thiosulfate, which neutralizes any residual chlorine in sample. Do not remove.
- Wearing disposable, thin plastic gloves is recommended. If you don't have gloves, avoid touching inside of bottle or Thio Bag with bare hands.
- Fill a 10 mL pipette with water sample and pour into plastic bottle or Thio Bag
- Add 90 mL sterile water to bottle or Thio Bag. Record sample details.

3. Add *E. coli* growth medium to sample

- Open growth medium pouch and add test bud to sample. Leave white desiccant in foil pouch.
- Do not touch growth medium with bare fingers or hands
- Dissolve medium in sample for 10-12 minutes
- The medium dissolves from its plastic carrier. When the medium is completely dissolved, the plastic carrier turns white or nearly white.

4. Pour sample into compartment bag

- Label bag or attach barcode asset tag to compartment bag
- Tear off perforated seam at top of bag
- Rub top and sides of bag together in each compartment to open so water easily runs into compartments
- Use white tabs at top of bag to pull open
- Slowly pour sample into bag while gently tilting and squeezing bag to distribute sample amongst five compartments
- Do not pour test bud carrier into compartment bag
- Fill evenly to the top of the fill line



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5. Seal compartment bag shut

- Attach seal clip across the bag above the fill line and below the compartment top openings. Place U-shape across width of the bag above liquid level along the fill line but below compartment openings. Snap rod-shaped part of the clip from other side of bag into U-shape to lock in place.
- Close the top of the bag with the yellow Whirl-Pak seal and then roll down the bag toward the seal clip

6. Incubation Period

- During the incubation period, CBTs can develop an odor. Place CBTs in another sealed plastic bag or container during the incubation period.
- Ambient temperature incubation works at 25°- 44.5°C
- The CBT works at variable temperatures. Constant temperature control in an incubator is not required but is recommended in cooler temperatures if available.
- Below 25°C, use a portable incubator or find a warm location at or above 25°C for incubation

Incubation Period Time and Temperature Recommendations:

35-44.5°C: Incubate 20-24 hours

31-34°C: Incubate 24-30 hours

25-30°C: Incubate 40-48 hours

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

7. Score and record test results

- Align compartments in correct sequence to Aquagenx MPN Table, hold bag up to read results
- Yellow/yellow-brown indicates negative (absence) compartment for *E. coli*
- Blue/blue-green indicates positive (presence) compartment for *E. coli*. Any trace of blue or blue/green in a compartment is considered positive, even just specks of blue/blue green color or just blue/blue green sediment at bottom of compartment
- Match color sequence of five compartments to one of 32 rows in MPN Table to obtain MPN test results
- Record test results

8. Decontaminate sample

- CBT Kit 10-Pack users add three chlorine tablets included in the kit to top of compartment bag. Seal bag with seal clip. Agitate sealed bag until chlorine dissolves.
- CBT Kit 50-Pack users source and add 1-2 mL of liquid bleach (NaOCl) or sufficient free chlorine tablets (calcium hypochlorite or sodium dichloroisocyanurate) to provide about 100 milligrams of free chlorine.
- After 45 minutes, pour contents into a sink, toilet or hole in ground and safely dispose the empty compartment bag
- Retain seal clip for reuse

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