



Aquagenx[®] GEL EC Kit Colony Forming Units (CFU) Test Instructions for Use: 1:10 Dilution for Surface and Recreational Waters

Overview

The Aquagenx GEL EC CFU Kit detects and quantifies *E. coli* (EC) in a 100 mL water sample. Its upper detection limit for surface and recreational waters is 500 *E. coli* per 100 mL. The kit can be used to analyze 100 mL water samples but it may be difficult to count *E. coli* colonies if colonies are over 500 CFU/100 mL.

The EC growth medium for *E. coli* is a proprietary chromogenic powder growth medium with a substrate mixture that detects β -glucuronidase. The GEL medium is a proprietary powder growth medium with a mixture of plant-based gelling materials. When *E. coli* metabolize Aquagenx's GEL media, *E. coli* appear as blue colonies in the sample. Colonies have the appearance of small dots or circles.

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

Dilutions guidance: <https://www.aquagenx.com/dilutions-gel-ec/>

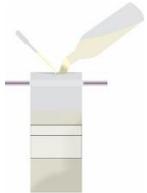
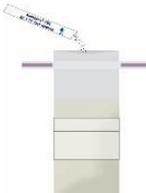
Shelf Life of Growth Media

Aquagenx GEL powder and EC growth medium are stable up to two-years after date of manufacture at 25° Celsius. Expiration date and lot number are printed on media packets.

Storage of Growth Media

Storage temperature is 10-25° Celsius in a dry environment. Growth media can be stored in a refrigerator. Cold chain for Aquagenx GEL powder and EC growth medium is not required.

Summary of Test Procedures for GEL EC CFU Kit with 1:10 Dilution

<p>Collect 10 mL sample, add 90 mL diluent</p> 	<p>Add EC powder growth medium to Thio-Bag</p> 	<p>Add GEL powder to GEL bag</p> 	<p>Pour sample from Thio-Bag into GEL bag</p> 
<p>Dissolve and spread GEL mixture. See Steps 4 and 5.</p> 	<p>Seal GEL bag shut</p> 	<p>Incubate at ambient temperature or in incubator. See Step 6.</p> 	<p>Count blue EC colonies in ambient light to score test results. Decontaminate sample.</p> 

How to Identify Color-Change for Colonies

E. coli = blue colonies (small dots or circles)

U.S. EPA 2012 Recreational Water Quality Criteria

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. SEE HOW-TO VIDEOS: <https://www.aquagenx.com/how-to-use-gel-ec/>

1. Prepare work area

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

2. Collect 10 mL water sample with Whirl-Pak™ Thio-Bag™ (since it will be used for surface water, Thio-bag is really necessary?)

- Wear disposable, thin plastic gloves.
- White tablet in Thio-Bag is sodium thiosulfate, which neutralizes residual chlorine in sample. Do not remove from bag.
- Fill a sterile 10 mL pipette with water sample and pour into Thio-Bag.

3. Add 90 mL of diluent to Whirl-Pak Thio-Bag

- Add 90 mL of *E. coli*-free water to Thio-Bag (up to 100 mL fill line). Record sample details.
- Diluent options: Standard Methods phosphate buffer; Autoclaved or boiled reagent water; Autoclaved or boiled tap water; High quality, certified bottled water. Some bottled waters are produced locally and are microbiologically unsafe; Distilled water from a commercial source and sold in properly sealed bottles. While not necessarily sterile, it should be free of *E. coli*.

4. Add Aquagenx EC 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.
- Tear downward on serrated edge on EC medium packet that is nearest to letters EXP. 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. Prepare larger GEL bag and add Aquagenx GEL powder

- Wear disposable, thin plastic gloves.
- Label larger GEL bag or attach barcode asset tag to bag.
- Hold the top of the GEL bag with both hands, pull apart the reclosable seal and shake bag to fully open the bag.
- Tear downward on serrated edge on larger GEL medium packet that is nearest to letters EXP. Pour powder growth medium into GEL Bag. Do not touch GEL powder with bare fingers or hands.
- Pour GEL powder into open bag. Gently tilt and shift the bag to move the powder out of the corners and to the center and bottom of the bag as much as possible.

5. Pour sample with dissolved EC medium from Thio-Bag into Aquagenx GEL bag

- Pour entire sample from Thio-Bag into fully opened GEL bag, but do not seal the bag shut yet. As soon as the sample is added, lie the GEL bag on flat surface with the bag opening slightly elevated so the mixture will not leak out of the bag.
- Firmly and rapidly use your hand to press the mixture toward the bottom of the bag. Massage and press clumps of powder to help them dissolve. Continue until mixture becomes thick and gelatinous.

- Use the palm of hand to spread and press the mixture toward the top of the bag. When the mixture is about 2/3 up the length of the bag, seal the bag shut.
- Continue spreading and pressing the mixture throughout the bag to achieve as uniform a thickness as possible.

6. Incubation Period and Temperatures

- Place each GEL bag on a flat surface. Do not stack more than two GEL bags on top of one another. Each stacked bag should align edge-to-edge around the perimeter to avoid the top bag drooping or hanging over the bottom bag. Move bags with care after the incubation period.
- Ambient temperature incubation works at any temperatures between 25°- 44°C for detection of *E. coli*.
- Because the GEL test works at variable temperatures, constant temperature control in an incubator is not required. However, at cooler temperatures, constant temperature incubation is recommended, if available.
- For regulatory compliance purposes, samples must be incubated at 35±0.5°C for 20-24 hours to detect and quantify *E. coli*. If possible, incubation at 44.5±0.5°C for 2-24 hours will obtain cleaner background and easy to count for *E coli* colonies when 100 mL sample is used.
- The GEL test also can be used to detect and quantify thermotolerant (or fecal) coliforms if the GEL samples are incubated at a temperature of 44.5°C (between 44-4°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 on a flat surface
31-34°C:	Incubate 24-30 hours on a flat surface
25-30°C:	Incubate 40-48 hours on a flat surface

Recommended Incubation Period Using an Incubator

35±0.5°C: Incubate 20-24 hours. When stacking GEL bags in an incubator, do not stack more than two bags on top of each other. Ensure bags are precisely aligned around the perimeter to avoid the top bag drooping or hanging over the bottom bag. Remove bags with care from the incubator after the incubation period.

7. Score CFU test results

- After appropriate incubation period, count the number of colonies in GEL bag in ambient light:
 - *E. coli* are blue colonies
 - Upper detection limit of GEL test for surface and recreational water of *E. coli* is 500 CFU/100 mL.

8. Decontaminate sample

- Add 4 mL of liquid bleach (NaOCl) or sufficient chlorine tablets (calcium hypochlorite or sodium dichloroisocyanurate) to GEL 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 GEL bag.