



**PRELIMINARY DRAFT April 2021**  
**Aquagenx® GEL EC Kit**  
**Colony Forming Units (CFU) Test**  
**Instructions for Use: Drinking Water**

**Overview**

The Aquagenx GEL EC CFU Kit detects and quantifies *E. coli* (EC) in a 100 mL water sample. Its upper detection limit for drinking water is 200-300 *E. coli* per 100 mL.

The EC growth medium for *E. coli* is a proprietary chromogenic powder growth medium with a substrate mixture that detects  $\beta$ -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/blue-purple colonies in the sample. Colonies have the appearance of small dots or circles. *E. coli* is a fecal coliform as well as a thermotolerant coliform.









**Shelf Life**

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

**Storage**

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

**Summary of Test Procedures for GEL EC Kit**

<p>Collect sample With 100 mL Thio-Bag</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 to score test results. Decontaminate sample.</p> 

**How to Identify Color-Change for Colonies**

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

World Health Organization (WHO) Guidelines for Drinking Water Quality, Table 5.4, Fourth Edition, 2017

		Sanitary inspection risk score (susceptibility of supply to contamination from human and animal faeces)			
		0-2	3-5	6-8	9-10
E.coli classification (as decimal concentration/100)	< 1				
	1-10				
	11-100				
	> 100				

Low risk: no action required	Intermediate risk: low action priority	High risk: higher action priority	Very high risk: urgent action required
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**Procedural Notes**

**1. Prepare work area**

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

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

- Wear disposable, thin plastic gloves.
- White tablet in Thio-Bag is sodium thiosulfate, which neutralizes residual chlorine in sample. Do not remove.
- Fill Thio-Bag to 100 mL fill mark. Record sample details.

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

- Open medium packet with scissors and pour powder growth medium into Thio-Bag. Growth medium should not be added to sample before you are ready to proceed with the entire testing procedure.
- Roll down Whirl-Pak seal and close Thio-Bag shut.
- Dissolve medium in sample. Gently swirl the bag until the medium is completely dissolved. Squeeze clumps of powder to help them dissolve more quickly.

**4. Prepare larger GEL bag and add Aquagenx GEL powder (see video on website)**

- Wear disposable, thin plastic gloves.
- Label larger GEL bag or attach barcode asset tag to bag.
- Hold the top of the bag with both hands, pull apart the reclosable seal and shake bag to fully open the bag.
- Open GEL packet with scissors and 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 (see video on website)**

- Pour entire sample from Thio-Bag into fully opened GEL bag, but do not seal the bag yet. As soon as the sample is added, squeeze the mixture with your fingers for a few seconds to begin to dissolve powder. Next, 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. You can massage and press clumps of powder to help them dissolve. Continue until mixture becomes thick and gelatinous.
- Next, 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 bag on a flat surface. Do not stack more than three bags on top of one another. Each stacked bag should align edge-to-edge around the perimeter to avoid the top bags drooping or hanging over the bottom bags. Move bags with care after the incubation period.
- Ambient temperature incubation works at any temperatures between 25°- 44.5°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*.
- 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 44-45 °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 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 three bags on top of each other. Ensure bags are precisely aligned around the perimeter to avoid the top bags drooping or hanging over the bottom bags. Remove bags with care from the incubator after the incubation period.

## 7. Score and record CFU test results

- After appropriate incubation period, count the number of colonies in GEL bag:
  - *E. coli* are blue/blue-purple colonies
  - Upper detection limit for drinking water of *E. coli* is 200-300 CFU/100 mL

	WHO Health Risk Category Drinking Water
<b>Number of colonies (CFU): <i>E. coli</i></b>	
<1	Low risk: no action required
1 - 10	Intermediate risk: low action priority
11 - 100	High risk: higher action priority
>100	Very high risk: urgent action required

## 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.