



Aquagenx® GEL AMR EC Kit Antimicrobial Resistant (AMR) ESBL *E. coli* Bacteria Colony Forming Units (CFU) Test Instructions for Use: Drinking & Surface Water

Overview

The Aquagenx GEL AMR EC CFU Kit detects and quantifies extended spectrum beta-lactam (ESBL) *E. coli* (EC) in a 100 mL water sample. It uses a proprietary powder growth medium with a glucose substrate and the antibiotic Cefotaxime to detect antibiotic resistant strains of *E. coli*, specifically those with ESBL resistance. The GEL Powder is a proprietary powder with a mixture of plant-based gelling materials. When *E. coli* metabolize the substrate in Aquagenx's growth medium, AMR *E. coli* appear as blue/blue-purple dots or small circles, indicating the presence of Cefotaxime resistant *E. coli*. CFU test results are obtained by counting the blue dots as colonies.

This test can be used for wastewater testing using a series of dilutions. See **Product Documents:** <https://www.aquagenx.com/product-documentation/>

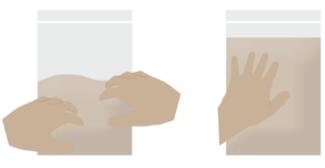
Shelf Life of Growth Media

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

Storage of Growth Media

Storage temperature is 4-25° Celsius in a dry environment. Growth media can be stored in a refrigerator. Cold chain for Aquagenx GEL powder and ESBL EC growth medium is not required.

Summary of Test Procedures for GEL EC CFU 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 in ambient light to score test results. Decontaminate sample.</p> 

How to Identify Color-Change for Colonies

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

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

3. Add Aquagenx ESBL 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 partially open the bag.
- Tear downward on serrated edge of larger GEL powder packet that is nearest to letters EXP. Pour GEL powder into middle of GEL bag. Do not touch GEL powder with bare fingers or hands.
- Thinly spread the powder across the middle of the bag by tilting the bag and spreading the powder with your fingers.

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

- Quickly pour the entire sample from Thio-Bag into the GEL bag, but do not seal the bag shut yet. IMMEDIATELY lay the GEL bag on a flat surface.
- QUICKLY AND FIRMLY use the palm of 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.
- When the powder is dissolved, spread the mixture in an even thickness toward the top of the bag.
- When the mixture is near the top of the bag, seal the bag shut.

6. Incubation Period and Temperatures

- During the incubation period, GEL tests can develop an odor. To help control odor, lie GEL bags flat in a sealed box or other container. Move GEL bags with care.
- Incubate GEL bags 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.
- Ambient temperature incubation works at any temperature between 25°- 37°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*.

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 four 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:
 - *ESBL E. coli* are blue colonies
 - Upper detection limit of GEL test for drinking and surface water of *ESBL E. coli* is 200-300 CFU/100 mL
 - Count and record results for number of *ESBL E. coli* colonies per 100 ml

8. Decontaminate sample

- Add 4 mL of liquid bleach (NaOCl) 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.