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High View Innovation

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E.coli & Total Coliform Detection Kit

Description:

E.coli & Coliform bacteria are the commonly used bacterial indicators of sanitary quality of food and water. They are defined as rodshaped Gram-negative non-spore forming bacteria which can ferment lactose with the production of acid and gas when incubated at 35-37°C. Our detection system utilizes a fluorogenic substrate which, when hydrolyzed by a specific enzyme (during peptide hydrolysis) produces a fluorescence which is read by a fluorometer.

Assay Performance:

- Rapid (30-min test plus incubation time), convenient, and sensitive. (Semi-quantitative if calibrated by user.)
- Highly portable field kit using handheld fluorometer for measurement.
- Sensitivity: 100 cfu/sampling after 8 hours of Incubation. 1 cfu/sampling after 10 hours of incubation.
- Can be applied for food surface, food-processing facility surface, or human/animal surface.

Required Equipment and Assay Kit (E.coli / Total Coliform):

- Dual-channel handheld fluorometer (E.coli & Total Coliform)
- E.coli handheld fluorometer
- Total Coliform handheld fluorometer
- E.coli Assay Kit (50 tests)
- Total Coliform Assay Kit (50 tests)

Content of the Assay Kit(50 tests):

- Reagent A (Substrate): 3.5 mL
- Reagent B (Enzyme Inducer): 2 mL
- Reagent C (Lysing Agent): 6 mL
- Incubation Media Powder: 0.8 g
- Large Plastic Vials (Sample): 50 pcs
- Small Plastic Vials (Mixing): 50 pcs
- Short Rayon Swab: 50 pcs

Other Materials Suggested:

- 5-setting Pipette
- Disposable Pipette Tip, 200-uL

Assay Procedure:

Information: In some instances the target organism might be stressed, and may not be producing the detectable enzyme. Therefore, a growth phase requiring incubation may be necessary. If testing is performed without any incubation and the result is negative, and a concern remains, then perform the 3-10 hour incubation phase which will allow the microorganism to begin producing detectable enzyme. Also, if you need to detect low levels of the target organism, anything below 250,000 cfu/sampling, then the 3-10 hours incubation phase is recommended.

I. For Preparing Samples Collected from Surface:

1. Prepare incubation media: Add 130 mg of Incubation Media Powder into 10-mL of distilled water in a sterilized container. Mix thoroughly. The media solution can be stored at 4° C for 1 week.
2. Pipette 1mL of incubation media (if incubated), or dH2O (if no incubated) into a Large Plastic Vial (Sample).
3. Add 1 drop of Reagent B (Enzyme Inducer) into the Large Plastic Vial (Sample).
4. Put a few drops of the incubation media on a sterile rayon swab, and collect the bacteria sample by swabbing the test area surface. (Note: follow proper swabbing techniques to obtain the optimum sample).
5. Place the swab tip into the Sample Plastic Vial. Agitate to mix the solution with the swab, and then squeeze the swab on the Vial wall to release as much liquid as possible. Secure the vial cap. **Go to Step 6.**

II. For Preparing Samples from Water Source:

1. Prepare concentrated incubation media solution: Add 150 mg of Incubation Media Powder into 1-mL of distilled water in a sterilized container. Mix thoroughly. The concentrated media solution can be stored at 4° C for 1 week.
2. Pipette 1 mL of water sample into a Large Plastic Vial (Sample), then add 0.1 mL of the concentrated incubation media solution into the Large Plastic Vial (Sample).
3. Add 1 drop of Reagent B (Enzyme Inducer) into the Large Plastic Vial (Sample). Secure the vial cap. **Go to Step 6.**

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III. Testing Procedures:

6. If incubated, put the Large Plastic Vial (Sample) at 38.5 °C for a minimum of 3-10 hours. If overnight incubation is used, up to 16-hour incubation can be done, but no more than 16-hour is preferred to reduce the possibility of false positive. (Semi-quantitative measurement requires incubation for 10 hours.)
7. Add 3 drops of Reagent C (Lysing Agent) into the Small Plastic Vial (Mixing).
8. Pipette 200-µL of the sample into the Small Plastic Vial (Mixing). Gently mix by pipetting 5-10 times.
9. Wait 5-6 minutes. In the meantime, turn on the Fluorometer to warm up the meter.
10. Add 2 drops of Reagent A (Substrate) into the Small Plastic Vial (Mixing). Secure the cap and gently mix by turning the vial upside down. Wait 1 minute.
11. Place the Small Plastic Vial (Mixing) into the Fluorometer test chamber and secure the cap on the test chamber. (Note: Wipe the outside of the test tube with a lint free cloth, and make sure there are no bubbles in the tube. Keep the test tube in the same direction.)

A. Qualitative measurement:

10. From the dual-channel fluorometer main screen, press the [Measure] button. → Measure E.coli, press [channel 1]/ Measure Total Coliform, press [channel 2]. (If you using the single channel E.coli or Total Coliform fluorometer, press [Assay 1].
11. Press the [Measure] button and write down the result number P1 as shown on the screen.
12. If P1 > 30,000, or the screen shows “Over Limit”, the Sample is positive and stop here. Otherwise, continue to the next step.
13. Don’t move the Small Plastic Vial (Mixing). The fluorometer will start to measure after waiting for 20 minutes. Write down the result number P2 as shown on the screen.
14. If the numerical value (P2-P1) > (7%xP1), or P2 is “Over Limit”, the Sample is positive.
15. If the numerical value (P2-P1) < (3%xP1), the Sample is negative.
16. If the value (P2-P1) is between (3%xP1) and (7%xP1), retest after another 20 minutes to get result number P3. If (P3-P1) > (7%xP1) the Sample is positive. Otherwise the Sample is negative.
17. You can test multiple samples by recording P1 or P2 value before changing to another sample. But the measurement after 20 minutes requires manual measurement to control the exact time.

B. Semi-qualitative measurement: (for reference only, applies to small dynamic ranges: 2~3 orders of magnitude CFU)

10. The process requires incubation for 10 hours (or the time according to the CFU table you build).
11. From the dual-channel fluorometer main screen, press the [Measure] button. → Measure E.coli, press [channel 1]/ Measure Total Coliform, press [channel 2]. (If you using the single channel E.coli or Total Coliform fluorometer, press [Assay 1].
12. Press [Blank]→[Measure]. The value on the screen should be close to zero.
13. Don’t move the Small Plastic Vial (Mixing). The fluorometer will start to measure after waiting for 20 minutes. Write down the result number P as shown on the screen.
14. Refer to (C) CFU table below; using the linear or logarithmic interpolation P to calculate the CFU estimated value.
15. During the test, keep the same temperature as the CFU table test to get the correct result.

C. Construction of a CFU table:

1. Start from the measurement procedure 1, and perform [Semi-qualitative measurement] procedure.
2. Add the known number of CFU into the Large Plastic Vial (Sample). It is recommended to use several 10x samples (e.g., 1, 10, 100, 1000 CFU).
3. Incubation for 10 hours (or according to the CFU measurement range) to get number P. That becomes the CFU table.
4. For each batch of new reagents, it is necessary to perform this construction of the CFU table process. The CFU table for each batch is valid for 3 months.

Recommendation: Every 20 minutes of incubation, the number of bacteria will be doubled. So it is important to control the incubation time.