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BactTest™ Dual-Channel Procedure for E.coli and Total Coliform

Description:

The BactTest convenient field detection kit utilizes a fluorogenic substrate which, when hydrolyzed by a specific enzyme of the target batercia (during peptide hydrolysis), produces a fluorescence which is read by our dual-channel handheld fluorometer.

Assay Performance:

- Rapid (30-min test plus incubation time), convenient, and sensitive.
- Highly portable field kit using handheld fluorometer for measurement.
- Sensitivity: 1 CFU/sample after 10 hours of incubation.
- If no incubation, 250,000 CFU/sampling is required for detection.
- Can be applied for drinking water, recreational water, beverages, and surface samples.

Required and Recommended Equipment and Assay Kit:

P/N	Description
BTD-ECOL/TCOL-B	BactTest Dual-Channel Handheld Fluorometer w/Power Supply
ECOL-SW50-ET	E.coli Detection Assay Kt, 50 tests
TCOL-SW50-ET	Total Coliform Detection Assay Kt, 50 tests
CC-215	Compact Carrying Case for Field Test

Content of each Assay Kit (PN: ECOL-SW50-ET or TCOL-SW50-ET):

Reagent A (Substrate): 3.5-mL
Reagent C (Lysing Agent): 4-mL
Large Plastic Vials (Sample): 50 pcs
(Store reagents at below -10°C)
Growth Media Powder: 800-mg
Small Plastic Vials (Testing): 50 pcs
Rayon Swab: 50 pcs

Assay Procedure:

Information: In some instances the target organism might be stressed, and may not be producing enough detectable enzyme. Therefore, a growth phase requiring incubation may be necessary to allow the micro-organism to produce detectable enzyme. Also, if you need to detect low levels of the target organism below 250,000 CFU/sample, the 3-10 hours incubation phase is recommended.

I. For Preparing Surface Samples:

1. If incubation is required, prepare incubation media by dissolving 130-mg of Growth Media Powder into every 10-mL of dH_2O in a sterilized container. The media solution can be stored at 4°C for up to 2 weeks.
2. Pipette 1-mL of incubation media (if incubated), or dH_2O (if no incubated) into a Large Plastic Vial (*Sample*).
3. Add two drops of solution from the Large Plactic Vial on a Rayon Swab, and collect the bacteria sample by swabbing the test area. (*Note: follow proper swabbing techniques to obtain the optimum sample*.)
4. Place the Rayon Swab tip into the Large Plastic Vial (*Sample*). Agitate to mix the solution with the swab, and then cut the handle of the swab to leave the swab tip in the Large Plastic Vial (*Sample*). Secure the vial cap. Go to Step 5.

II. For Preparing Water Samples:

1. Note: In order to avoid interference from unknown fluorescent substances in the water sample, we recommend you take a fluorometer reading on the unprepared water sample first. Go to the channel as shown in Step 8, and measure the water sample inside a Small Plastic Vial. If the reading is higher than 1000, the following bacteria measurement results may be unreliable.
2. Re-constitute the growth media solution: Add 150-mg of Growth Media Powder into every 1-mL of distilled water in a sterilized container. Mix thoroughly. The media solution can be stored at 4°C for up to 2 weeks.
3. Pipette 1-mL of water sample into a Large Plastic Vial (*Sample*). If incubation is desired, add 0.1-mL of growth media solution into the vial. Mix the content by inversion. Go to Step 5.

III. Testing procedures:

5. If incubated, put the Large Plastic Vial (*Sample*) at 38°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.
6. Obtain 1 Small Plastic Vial (*Testing*) from the kit, and add 2 drops of Reagent C (Lysing agent) into the vial.
7. With a disposable pipette or pipette tip, pipette 200-μL of the *Sample* into the Small Plastic Vial (*Testing*). Gently mix by pipettinig.
8. Wait 5-6 minutes. In the mean time, turn on the fluorometer to warm up the meter. From the main screen, Press [Measure], then select the desired channel:

   For E.coli: select [E. Coli]
   For Total Coliform: select [T. Coliform]
9. Add 2 drops of Reagent A (Substrate) into the Small Plastic Vial (*Testing*), Gently mix by inverting the vial.
10. Place the Small Plastic Vial (*Testing*) into the Fluorometer test chamber and close the cap on the test chamber. (*Note: Wipe the outside of the vial with a lint free cloth, and make sure there are no bubbles in or at the bottom the vial.*)
11. Press the [**Measure**] button immediately and write down the result number P1 as shown on the screen.
12. If P1 > 5,000, or the screen shows "Over Limit", the *Sample* is **positive** and stop here. Otherwise, continue to the next step.
13. Wait for 20 minutes. The fluorometer will automatically take another measurement. Write down the result number P2.
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%-7% of P1, 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 you will have to manually repeating the [Return] → [Measure] buttons to take the measurement manually before and after the 20 minutes wait time. Make sure to place the vials in the same orientation and securely each time to maintain the signal consistency.