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Product Information

***Fluoro*SELECT™ Gram Negative Assay Kit**

Catalog Number 91333

Storage Temperature 2-8 °C

Product Description

Gram-negative bacteria are bacteria that do not retain crystal violet dye in the Gram staining protocol. Compared with Gram-positive bacteria, Gram-negative bacteria are more resistant against antibiotics, because of their impenetrable wall. 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 at $360nm_{ex}/460nm_{em}$.

- Rapid (30-min test plus incubation time), convenient, and sensitive.
- High portability using handheld fluorometer for measurement.

Components

- Reagent A (Substrate): min. 4 mL
- Reagent B (Enzyme Inducer): min. 2 mL
- Reagent C (Lysing Agent): min. 6 mL
- Incubation Media Powder: 0.4 g
- Large Plastic Vials (Sample): 50 pcs
- Small Plastic Vials (Mixing): 50 pcs
- Mini Glass Tubes (Testing): 50 pcs.

Equipment and Material Required but not Provided:

- *Fluoro*SELECT™ single channel fluorometer (λex 360 nm; λem 460 nm)
- Sterile rayon swab
- Distilled H_2O
- Pipette and pipette tip

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability/Safety

The kit is shipped at room temperature. Shelf life is 12 months. Safety procedures include wearing safety glasses, gloves and protective clothing.

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.

Detailed protocol:

1. If incubation is required, prepare incubation media by dissolving 0.013 g of Incubation Media Powder into every 1 mL of distilled H_2O.
2. Pipette 0.5mL of incubation media (if incubated), or distilled H_2O (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. Using a sterile rayon swab, collect the bacteria sample by swabbing the test area (*Note: follow proper swabbing techniques to obtain the optimum sample*).
5. Place the swab tip into the Large Plastic Vial (*Sample*). Agitate to mix the solution with the swab, and then break the handle of the swab by bending the swab shaft before putting the swab tip into the Large Plastic Vial (*Sample*). Secure the vial cap.
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.
7. Obtain 1 Small Plastic Vial (*Mixing*) from the kit, and add 3 drops of Reagent C (Lysing agent) into the vial.
8. With a disposable pipette or pipette tip, pipette 200 µL of the *Sample* into the Small Plastic Vial (*Mixing*). Gently mix by pipettinig 5-10 times.
9. Wait 5-10 minutes. In the mean time, turn on the Fluorometer to warm up the meter.
10. Add 2 drops of Reagent A (Substrate) into the Small Plastic Vial (*Mixing*), Gently mix by pipetting 5-10 times.

11. Prepare 1 Small Glass Tube (*Testing*), and pipette 200 µL of solution from the Small Plastic Vial (*Mixing*).
12. Wait 3- 5 minutes.
13. Place the Small Glass Tube (*Testing*) into the Fluorometer test chamber and secure the cap on the test chamber. (*Note: Wipe the outside of the glass tube with a lint free cloth, and make sure there are no bubbles in the tube.*)
14. From the Fluorometer main screen. Press [Measure] → [Next] → [RFU Lo].
15. Press the [Measure] button and write down the result number P1 as shown on the screen.
16. If P1 > 30,000, or the screen shows "Over Limit", the *Sample* is **positive** and stop here. Otherwise, continue to the next step.
17. Waite for 20 minutes. Press the [Measure] button and write down the result number P2 as shown on the screen.
18. If the numerical value (P2-P1) > (6%xP1), or P2 is "Over Limit", the *Sample* is **positive**.
19. If the numerical value (P2-P1) < (3%xP1), the *Sample* is **negative**.
20. If the value (P2-P1) is between 3%-6% of P1, retest after another 20 minutes to get result number P3. If (P3-P1) > (6%xP1) the *sample* is **positive**. Otherwise the *Sample* is **negative**.
21. You can test multiple samples by recording P1 or P2 value before changing to another sample.

Short protocol:

1. A cotton swab is used to wipe a particular surface of the detection area. The swab is then inserted into 0.5mL of distilled water for an immediate test, or into 0.5mL of incubation solution for an incubation period ranging from 3 to 10 hours at 38.5°C, before measuring. Add an enzyme inducer after inserting the swab into the water or incubation solution.

2. Then add a lysing agent to the solution and let incubate for 5 minutes.

3. Next, a substrate is added to the solution and allowed to stand for 5 minutes.

4. Afterwards, the solution is transferred into a glass test tube and then inserted into the Fluorometer. The first measurement is taken immediately, and after 20 minutes, another measurement is taken.

Assay performance

- Detection range: 250,000 CFU/sampling, immediately showing result.
- 100 cfu/sampling after 8 hours of Incubation.
- 1 cfu/sampling after 10 hours of incubation.

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