高識能股份有限公司
High View Innovation

No.251, Niupu Road, Hsinchu City 300, Taiwan
Tel: 03-5384063
http://www.hviglobal.com

Gram Negative Detection Field Kit for Surface

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 360ex/460em.

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:

Handheld Fluorometer
Gram Negative Detection Assay Kit for Surface, 50 tests

Other Materials Suggested:

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

Content of the Assay Kit (50 tests):

Reagent A (Substrate): min. 4 mL ； Reagent B (Enzyme Inducer): min. 2 ； Reagent C (Lysing Agent): min. 6 mL ； Large Plastic Vials (Sample): 50 pcs ； Small Plastic Vials (Mixing): 50 pcs ； Short Rayon Swab: 50 pcs ； Incubation Media Powder: 0.4 g

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.

01. If incubation is required, prepare incubation media by dissolving 0.013-g of Incubation Media Powder into every 1-mL of dH2O.
02. Pipette 600-µL of incubation media (if incubated), or dH2O (if no incubated) into a Large Plastic Vial (*Sample*).
03. Add 1 drop of Reagent B (Enzyme Inducer) into the Large Plastic Vial (*Sample*).
04. Using a sterile rayon swab, collect the bacteria sample by swabbing the test area (Note: follow proper swabbing techniques to obtain the optimum sample).
05. Place the swab tip into the Large Plastic Vial (*Sample*). Stir 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.
06. 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.
07. Obtain 1 Small Plastic Vial (*Mixing*) from the kit, and add 3 drops of Reagent C (Lysing agent) into the vial.
08. 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.
09. Wait 5-10 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*). Close the cap and gently mix by shaking the vial.
11. Wait 1 minute.
12. 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.)
13. From the Fluorometer main screen. Press [Measure] → [Next] → [RFU Lo].
14. Press the [Measure] button and write down the result number P1 as shown on the screen.
15. If P1 > 30,000, or the screen shows “Over Limit”, the *Sample* is **positive** and stop here. Otherwise, continue to the next step.
16. Wait for 20 minutes. Press the [Measure] button and write down the result number P2 as shown on the screen.
17. If the numerical value (P2-P1) > (6%xP1), or P2 is “Over Limit”, the *Sample* is **positive**.
18. If the numerical value (P2-P1) < (3%xP1), the *Sample* is **negative**.
19. 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**.
20. You can test multiple samples by recording P1 or P2 value before changing to another sample.