

Now! Test Negative Control Protocol

- 1) Don proper PPE and follow aseptic technique as a measure of good practice.
- 2) Draw up 0.5 mL of supplied pre-packaged sterile water (emptied in the provided ziplock bag or a sterile container, e.g. urine cup) using the supplied graduated pipette.
- 3) Add the water to the provided cuvette with the growth medium.
- 4) Tightly close the cuvette lid and invert gently a couple times for mixing.
- 5) Place vials in the block incubator and allow 12 or more hours of incubation. The incubator should be set to 37°C.
- 6) After incubation, the cuvette needs to be allowed to cool down. One of two methods can be employed for cooling down the sample:

Cooling Sample

- a. Room temperature cooling: remove the cuvette and place in the supplied holder and allow cooling for a minimum of **1 hour**, but not greater than 3 hours.
Continue to the next step.
 - b. Refrigerator cooling: remove the cuvette and place in the supplied holder. Place in a refrigerator (approximate temperature of 4°C) for **15 minutes**. Remove from the refrigerator at 15 minutes and continue to the next step.
- 7) Before adding Reagent A, switch the power source of the fluorometer at the upper right corner to “ON”.
 - 8) Add 2 drops of Reagent A to the cuvette.
 - 9) ‘Gently’ invert the cuvette about four times to help mix the Reagent A with the sample. While inverting hold the cuvette at its top half. Make sure there are no air bubbles. Immediately proceed to the next steps for testing.
 - 10) Place the cuvette in the fluorometer, line up the pointy side of the cuvette with the black line in the reader, and place the black cap firmly on the fluorometer.
 - 11) Press the “Measure” button on the fluorometer.
 - 12) Press “Blank” (timer will start counting seconds).
 - 13) Press “Measure” and wait 10 minutes to get the reading.

14) At 10 minutes, the fluorometer will automatically take a reading. (A value will be displayed in the box below the timer). The value displayed before 10 minutes is disregarded. Please Note: The timer on the fluorometer will continue to run, but the reading displayed is taken exactly at the 10 minute mark.

15) To begin testing a new sample, press "Return" twice to return to the main menu of the fluorometer.

INTERPRETATION OF RESULTS

- A numerical value should be less than "200."
- A value greater than "200" may indicate:
 1. The cuvette was not allowed to cool long enough (Step 6).
 2. The sample was contaminated during testing with Gram-negative bacteria.
 3. Bubbles were present when the cuvette was placed in the fluorometer and caused auto-fluorescence.
 4. There is an issue with either the consumables of the NOW! Test or the fluorometer.
- If the result is greater than 200, run a second negative control.
- If a result greater than 200 is again reported, contact Healthmark.