

PRIOR TO STARTING THE TEST:

Note: Food Samples should be prepared and enriched, following the directions outlined in the GeneQuence *Listeria* spp., test kit insert.

UPON OPENING THE KIT:



Add 12 mL of **pretreatment buffer** (bottle 1b) directly to one bottle of **pretreatment concentrate** (bottle 1a).



Add 12 mL of **lysis reagent buffer** (bottle 2b) directly to one bottle of **lysis reagent concentrate** (bottle 2a).



Mix **wash solution** (bottle 5) with 950 mL of distilled or deionized water.



STEP 1

For each sample and control, label a 12 x 75 mm glass tube with appropriate sample designation.



STEP 2

Place the appropriate number of microwells in the plate frame. Include first well for reagent blank, second well for negative control, and third well for positive control.

TEST PROCEDURE:

Add 0.4 mL of the samples and controls to the appropriately labeled tubes. Next, follow the steps below.



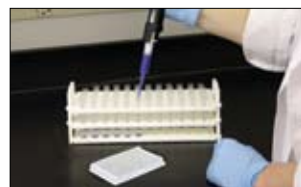
TEST STEP 1

Add 0.1 mL of **reconstituted pretreatment reagent** (bottle 1a) and 0.1 mL of **reconstituted lysis reagent** (bottle 2a) to each tube, or add 0.2 mL of the combined **pretreatment/lysis solution** (bottle 2a) to each tube.

Incubate the rack of tubes in the 37°C water bath or heater block for 5 minutes.



While the rack is incubating, refer to chart and mix **hybridization solution** (bottle 3) and **probe solution** (bottle 4) in a plastic or glass vial. Mix thoroughly.



TEST STEP 2

Transfer 0.150 mL of each lysed sample, including the controls, to designated microwells.



TEST STEP 3

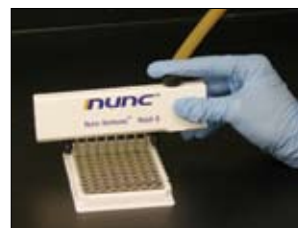
Add 0.125 mL of **hybridization/probe mixture** to each microwell, with the exception of the reagent blank microwell.

Incubate the plate at 45°C for 60 minutes.



TEST STEP 4

Wash the microwells 5 times.



TEST STEP 5

Add 0.150 mL of **substrate chromagen solution** (bottle 6) to each microwell, including the blank microwell.

Incubate the plate at room temperature for 20 minutes.



TEST STEP 6

Add 0.050 mL of **stop solution** (bottle 7) to each microwell, including the blank microwell.

Read absorbance at 450 nm using a plate strip reader.