soleris®

Direct Pseudomonas

Product Number: PD-109

Pictured: PD-109 vial uninoculated (left) and inoculated vial (right).

Procedure for Pseudomonas aeruginosa

The Direct *Pseudomonas* Vial (PD-109) with the addition of the supplement (S2-PSI) is specific for the detection of *Pseudomonas aeruginosa* in nutraceutical products. The vial has an assay time of 20 hours for most applications. This vial utilizes CO_2 for detection. The vial contains a selective medium and an antibiotic supplement is added to the vial. As organisms grow in the broth medium, the carbon dioxide (CO_2) produced diffuses through a membrane layer into a soft agar plug containing a dye indicator. The membrane layer also serves as a barrier, eliminating product interference with the reading window. The CO_2 released during the organism growth changes the agar plug from green/blue-green to yellow. The color change in the dye is read by the instrument.

Materials Required

1. PD-109, *Pseudomonas* Vial (PD-109)
2. Tryptic Soy broth (BLX-TSB90)
   a. If required, use a designated neutralization broth, such as D/E Neutralizer, TAT Broth, Modified Letheen Broth, etc.
3. S2 Pseudomonas Supplement (S2-PSI)
4. Oxidase Strips (BLX-OX)
5. Mini centrifuge
6. Microcentrifuge tubes

Dependent on Sample Tested

1. Sterile 1 N to 5 N sodium hydroxide (NaOH) and/or hydrochloric acid (HCl)
2. pH meter or pH paper

Vial Specifications

1. Vial pH is 7.0 ± 0.2
2. Vial sample capacity: 0.1 mL

Sample Preparation

1. For USP testing, perform 1:10 dilution by adding 10 g of sample in 90 mL of Tryptic Soy Broth (See NEOGEN Rapid Microbiology System Validation Book, Introduction, p.5) or designated neutralization broth.
   a. Check pH and adjust, if necessary, to 7.0 ± 1.0
2. Incubate for 18–24 hours at 35°C.

Vial Preparation

1. Remove PD-109 vials from the refrigerator and allow to equilibrate to room temperature

Inoculation of Vial

1. Transfer 0.1 mL of the S2 *Pseudomonas* Supplement (S2-PSI) to the PD-109 vial.
2. Cap the vial and gently invert 3 times to mix sample.
3. Transfer 0.1 mL of the incubated enrichment to the PD-109 vial.
4. Cap the vial and gently invert 3 times to mix sample. Keep cap tight.
5. Insert the vial into the Soleris® instrument set at 35°C and run for the pre-programmed test duration. It is not recommended to adjust the parameters without consulting NEOGEN Technical Services.
6. If detection occurs, perform the oxidase confirmation test.

Algorithm utilized:

Test	Threshold	Skip	Shuteye	Test Duration	Temperature
PD-109	10	1	35	20 hours	35°C

Oxidase Test Confirmation Procedure

1. Remove the PD-109 vial positive (detecting) vial from the instrument.
2. Transfer 1.0 mL from a positive PD-109 vial into a mini centrifuge tube.
3. Centrifuge for a minimum of 5 minutes or until a pellet forms.
4. After centrifugation, dispose of all the liquid from the tube.
   a. Repeat steps 2–4 until a pellet forms.
5. Place oxidase test strip in a petri dish and moisten an area of the strip to be tested with water. Do not saturate the strip. With a platnium loop, plastic loop or a toothpick, pick a little mass of the pellet, and put onto the oxidase paper.
6. After 20 seconds, a blue dot is an indication of positive results, indicating the presence of *Pseudomonas aeruginosa*.
7. Reference the kit insert for interpretation of results.
8. If positive, send out the sample for identification.

Disclaimers:

Information provided is based on validation procedures that NEOGEN performed in NEOGEN laboratories. Deviation from procedures is possible, but should be discussed with NEOGEN Technical Services.

Samples may need to be pH adjusted for all vials.

Appearance of the vials should be inspected prior to use.

Certain product matrices may require parameter adjustments, including increased test duration. For more information contact NEOGEN Technical Services.

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