Pseudomonas Broth, 2 mL
(6540)

Intended Use

Ampouled Pseudomonas Broth, 2 mL is a selective medium used for enumerating Pseudomonas aeruginosa and other Pseudomonas spp. in water and in various other applications by the membrane filtration method.

Product Summary and Explanation

Ampouled Pseudomonas Broth, 2 mL is a prepared, ready to use medium for membrane filtration testing. This medium is a modification of King's A Medium[1] and used for the detection and enumeration of pseudomonads in water and in other applications where membrane filtration methods are used.

Principles of the Procedure

Enzymatic Digest of Gelatin provides nitrogen, carbon, and minerals in Pseudomonas Broth. Magnesium chloride and Potassium sulfate enhance the production of pyocyanin and fluorescein dyes elaborated by some pseudomonads.[1] The CFC Supplement is a selective supplement used to inhibit Gram-positive organisms and Gram-negative bacteria other than Pseudomonas species.

Medium Composition: Per Liter

Enzymatic Digest of Gelatin	20.0 g
Magnesium chloride	1.4 g
Potassium sulfate	10.0 g
CFC Supplement	70.0 mg

Final pH: 7.1 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Physical Characteristics

Appearance of medium:	Clear to slightly hazy with no to trace precipitate, pale to light yellow
pH at 25ºC:	7.1 ± 0.2

Test Procedure

Preparation

1. Assemble the manifold or filtration flask that will supply the vacuum source, complete with rubber stopper.
2. Using a gentle twisting motion, secure the funnel adapter into the rubber stopper.
3. Using the same gentle twisting motion, secure the Neogen Filter onto the funnel adapter.

Filtration Procedure

1. Remove filtration cover and carefully pour the sample onto the filter.
2. Apply vacuum just long enough to pull the sample through the filter (if using a manifold, open only one valve at a time.)
3. Rinse the inside walls of the filter funnel with approximately 20 mL of sterile buffered solution. Apply vacuum just long enough to pull the solution through the filter, and turn off vacuum. Note: This step is optional if only water is being tested.
4. Briefly remove the filter and its funnel adapter from the rubber stopper to release any remaining vacuum pressure, and then re-secure into the stopper.
5. Add the Pseudomonas Broth onto the top of the filter. When doing so, be careful not to touch the filter with the tip of the ampoule.
6. Very briefly apply vacuum so that the media does not pool on top of the filter, and is visible underneath the filter. (Note: The media has been soaked correctly into the filter if there is a small pocket of air around the bottom port. The filter should be moist, but not oversaturated or dry.)
7. Remove and appropriately discard the plastic funnel. Place the filtration system cover over the filter/base assembly converting the unit to a Petri dish for sample incubation.

8. Remove the filter from the funnel adapter, and place a plug on the open bottom port.
9. Place the filtration plate into the incubator inverted so that the cover is on the bottom, and incubate at the appropriate temperature for isolation of the target Pseudomonas strains. See Limitations of the Procedure #4. Read and record results after 40 – 48 hours.
10. Dispose of test materials in accordance with all applicable local, state, and federal regulations.

Expected Cultural Response:
Sterile water was added to sterile filtration units and inoculated with the cultures listed below. The inoculum was filtered followed by the ampoulized Pseudomonas Broth and the filtration housing removed. Plates were incubated aerobically at 35 ± 2ºC for the P. aeruginosa strains and at 30 ± 2ºC for the P. fluorescens strain and examined for growth and reactions at 24 – 48 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results Examine at 18-24 hr. and at 40-48 hr.
Uninoculated Media	NA	No Growth
Pseudomonas aeruginosa ATCC 27853	10 - 300	≥ 85% recovery, beige w/ a slight green hue; blue to green fluorescence at 365nm
Pseudomonas aeruginosa ATCC 10145	10 - 300	≥ 85% recovery, green colonies; blue to green fluorescence at 365nm
Pseudomonas fluorescens ATCC 13525	10 - 300	≥ 85% recovery, off-white to beige colonies; blue to green fluorescence at 365nm
Escherichia coli ATCC 25922	300 -1000	Suppressed to inhibited
Proteus mirabilis ATCC 12453	300 -1000	Suppressed to Inhibited

Results
Examine filters for the presence of green, blue or blue-green colonies indicating presumptive isolation of P. aeruginosa. Examine filters for the presence of off-white to beige colonies indicating presumptive isolation of P. fluorescens. Examination of the presumptive positive pseudomonads under long wavelength UV light (365nm) will further identify the fluorescing species such as P. aeruginosa and P. fluorescens. Report the pseudomonads density in terms of total pseudomonads/100 mL. Non-pseudomonads or non-dye-producing pseudomonads may form colorless to straw-colored colonies where recovered.

Storage
Store ampouled Pseudomonas Broth, 2 mL at 2 - 8 ºC.

Expiration
Refer to expiration date printed on the front of the box container.

Limitations of the Procedure
1. Analyze sample as soon as possible after collection.
2. Samples containing colloidal or suspended particulate material can clog the membrane filter, thereby prevent filtration, or cause spreading of bacterial colonies which could interfere with colony identification.
3. If the inoculum is too heavy, differentiation of target colonies may be confusing since any dyes elaborated into the medium may spread underneath any other recovered bacterial colonies.
4. Clinical specimens may be recovered at 35 ± 2°C while environmental isolates or psychrotrophs may be recovered at 20-32°C.

Packaging
Pseudomonas Broth, 2 mL	**Code No.**	**6540**	**Box of 50**
Neogen Filter "White"	**Code No.**	**6550**	**Box of 50**
Neogen Filter "Black"	**Code No.**	**6555**	**Box of 50**

References

1. **King E.O., Ward M.K. and Raney D.E.** (1954) *J. Lab. & Clin. Med.* 44, 301-307

Technical Information

Contact Neogen Corporation for Technical Service or questions involving Ampouled Media at (517)372-9200 or (800)-234-5333 or fax us at (517)372-2006.

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