Pseudomonas Broth, 2 mL

Product Number: 6540

Medium Composition	
Enzymatic Digest of Gelatin	20.0 g
Magnesium Chloride	1.4 g
Potassium Sulfate	10.0 g
CFC Supplement	70.0 mg
Final pH: 7.2 ± 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 25°C: 7.1 ± 0.2

Intended Use

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

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.

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 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.

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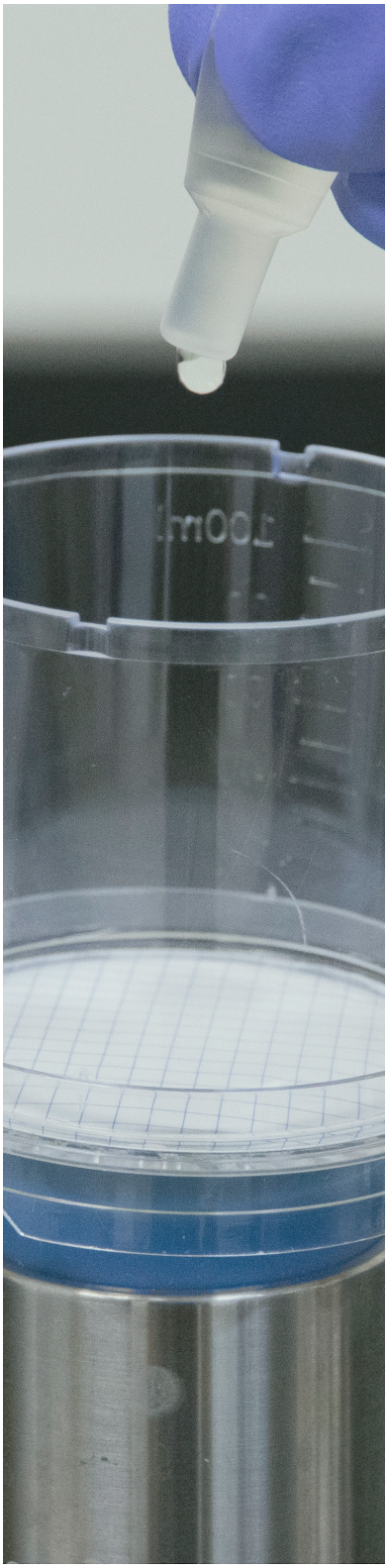

4. Briefly remove the filter and its funnel adapter from the stopper to release any remaining vacuum pressure, and then resecure into the stopper.
5. Add *Pseudomonas* Broth onto the top of the filter. When doing so, be careful not to touch the filter with the tip of the ampoule.
6. Very briefly apply 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 note 4 under the limitations of the procedure. 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 ± 2°C for the *P. aeruginosa* strains and at 30 ± 2°C for the *P. fluorescens* strain and examined for growth and reactions at 24–48 hours.

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Microorganisms	Approx . Inoculum (CFU)	Expected Results*
Uninoculated Media	N/A	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	300–1,000	Suppressed to Inhibited
Proteus mirabilis — ATCC 12453	300–1,000	Suppressed to Inhibited

** Examine at 18–24 hour and at 40-48 hour.*

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 ultraviolet light (365 nm) 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°C.

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.

NEOGEN Items		
6540	Pseudomonas Broth, 2 mL	Box of 50
6550	NEOGEN Filter — White	Box of 50

References

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

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