Orange Serum Broth, 2 mL

Product Number: 6525

Medium Composition	
Orange Serum	200 mL
Yeast Extract	3 g
Enzymatic Digest of Casein	10 g
Dextrose	4 g
Potassium	2.5 g
Final pH: 5.6 ± 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 or without a precipitate, and light to medium amber
pH at 25°C: 5.6 ± 0.2

Intended Use

Orange Serum Broth 2 mL is used for the cultivation of aciduric microorganisms associated with spoilage of products in membrane filtration procedures.

Product Summary

Orange Serum Broth is ready to use ampouled medium used in membrane filtration testing. Orange serum agar was developed for the isolation, cultivation, and determination of acid-tolerant spoilage organisms in fruit juices, fruit extracts, and citrus concentrates. The low pH of fruit juices makes citrus fruit products susceptible to spoilage by fungal and bacterial microorganisms.[1] Murdock, Folinazzo, and Troy[2] studied orange serum agar, pH 5.4, with *Leuconostoc*, *Lactobacillus*, and yeasts. The preparation of dehydrated media containing orange serum was described by Stevens.[3]

Principles of the Procedure

Enzymatic digest of casein provides carbon and nitrogen sources for general growth requirements. Orange serum provides the acid environment favorable to recovering acid-tolerant microorganisms. Yeast extract supplies B-complex vitamins that stimulate growth. Dextrose is the fermentable carbohydrate. Potassium phosphate is a buffering agent.

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.

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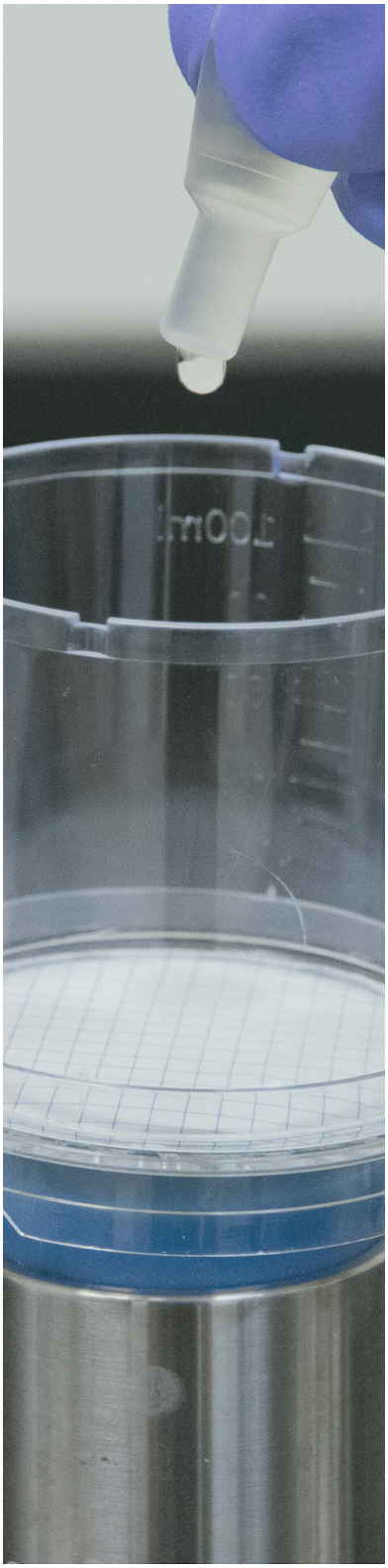

Filtration Procedure

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 stopper to release any remaining vacuum pressure, and then resecure into the stopper.
5. Add Orange Serum 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 incubated at 25–35°C. Read and record results after 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 ampoulized Orange Serum Broth and the filtration housing removed. Plates for acetobacter and yeast and molds strains were incubated aerobically at 25–30°C, plates for the Leuconostoc strain was incubated aerobically at 20–25°C, plates for *Lactobacillus* spp. (Lp & La) were incubated aerobically at 30–35°C, and plates for *Lactobacillus* spp. (Lc & Lf) were incubated in 5% CO2 at 35°C. Plates were examined for growth after 48 hours incubation.

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Microorganisms	Approx . Inoculum (CFU)	Expected Results
Uninoculated Media	N/A	No Growth
Acetobacter pasteurianus — ATCC 12879	10–100	≥ 85% Recovery
Aspergillus niger — ATCC 16404	10–100	≥ 85% Recovery
Candida albicans — ATCC 10231	10–100	≥ 85% Recovery
Lactobacillus acidophilus — ATCC 4356	10–100	≥ 85% Recovery
Lactobacillus casei — ATCC 393	10–100	≥ 85% Recovery
Lactobacillus fermentum — ATCC 9338	10–100	≥ 85% Recovery
Lactobacillus plantarum — ATCC 8014	10–100	≥ 85% Recovery
Leuconostoc mesenteroides — ATCC 12291	10–100	≥ 85% Recovery
Penicillium rocquefortii — ATCC 10110	10–100	≥ 85% Recovery
Saccharomyces cerevisae — ATCC 9763	10–100	≥ 85% Recovery

Results: Count all colonies that grow on the surface of the membrane and record.

Storage: Store Ampouled Orange Serum 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. Because bacteria found in bottled water demonstrate a prolonged lag phase during adaptation to growth on Orange Serum Broth, extended incubation beyond 48 hours may be required.

NEOGEN Items		
6525	Orange Serum Broth, 2 mL	Box of 50
6550	NEOGEN Filter — White	Box of 50
6555	NEOGEN Filter -— Black	Box of 50

References

1. Vanderzant, C., and D. F. Splittstoesser (eds.). 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C
2. Murdock, D. I., J. F. Folinazzo, and V. S. Troy. 1952. Evaluation of plating media for citrus concentrates. Food Technol. 6:181-185.
3. Stevens. 1954. Food Technol. 6:181.

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