Orange Serum Agar
Orange Serum Broth Concentrate 10×

Intended Use

Orange Serum Agar is used for cultivating aciduric microorganisms, particularly those associated with spoilage of citrus products.

Orange Serum Broth Concentrate 10× when diluted 1:10 is used for cultivating and enumerating microorganisms associated with spoilage of citrus products.

Summary and Explanation

The low pH of fruit juices makes citrus fruit products susceptible to spoilage by yeasts, molds and the bacteria *Lactobacillus* and *Leuconostoc*.[1] In the 1950s, Hays investigated spoilage in frozen concentrated orange juice. He found that an agar medium containing orange serum (juice) was superior to Lindegren Agar in isolating the microorganisms responsible for spoilage causing a buttermilk off-odor.[2] In a later comparative study, Murdock, Folinazzo and Troy found Orange Serum Agar, pH 5.4 to be a suitable medium for growing *Leuconostoc*, *Lactobacillus* and yeasts.[3] Stevens described preparation of dehydrated agar media containing orange serum.[4] The **BBL** formula for Orange Serum Agar differs only in a slightly increased orange serum content and in the incorporation of less agar.

Orange Serum Agar is included in recommended methods for examining fruit beverages.[1] Orange Serum Broth Concentrate 10× is used for small samples to initiate growth of saprophytic and pathogenic fungi,[5] as well as detecting and enumerating butyric acid anaerobes.[1]

Principles of the Procedure

Orange Serum Agar and Orange Serum Broth Concentrate 10× contain peptone as a source of carbon and nitrogen for general growth requirements. Orange serum provides the acid environment favorable to recovering acid-tolerant microorganisms. Yeast extract supplies B-complex vitamins which stimulate growth. Dextrose is the carbohydrate. Agar is the solidifying agent in Orange Serum Agar.

Formulae

BBL™ Orange Serum Agar

Approximate Formula* Per Liter

Orange Serum	10.0	g
Yeast Extract	3.0	g
Pancreatic Digest of Casein	10.0	g
Dextrose	4.0	g
Dipotassium Phosphate	2.5	g
Agar	15.5	g

Difco™ Orange Serum Broth Concentrate 10×

Approximate Formula* Per Ampule (100 mL)

Orange Serum	100.0	mL
Yeast Extract	30.0	g
Pancreatic Digest of Casein	100.0	g
Dextrose	40.0	g
Dipotassium Phosphate	25.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Orange Serum Broth Concentrate 10×

Concentrate Appearance:	Dark amber, clear solution.
Reaction of Solution at 25°C:	pH 5.6 ± 0.2

Cultural Response

Difco™ Orange Serum Broth Concentrate 10×

Prepare the medium per label directions. Inoculate tubes and incubate for 18-48 hours 35 ± 2°C for *Lactobacillus fermentum* and 30 ± 2°C for the remaining organisms.

ORGANISM	ATCC™	INOCULUM CFU	RESULT
Aspergillus brasiliensis (niger)	16404	30-300	Growth
Lactobacillus fermentum	9338	30-300	Growth
Leuconostoc mesenteroides	23386	30-300	Growth
Saccharomyces cerevisiae	9763	30-300	Growth

Identity Specifications

BBL™ Orange Serum Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain dark tan particles.
Solution:	4.5% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan; clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan; clear to slightly hazy.
Reaction of 4.5% Solution at 25°C:	pH 5.5 ± 0.2

Cultural Response

BBL™ Orange Serum Agar

Prepare the medium per label directions. Inoculate streak plates with fresh cultures and incubate for 66-72 hours at 30-32°C; for *Penicillium roquefortii*, incubate at 23-27°C. Inoculate pour plates with *Lactobacillus plantarum* and incubate for 66-72 hours at 30-32°C.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Lactobacillus gasseri	4962	Undiluted	Good
Leuconostoc mesenteroides	12291	Undiluted	Good
Penicillium roquefortii	10110	Undiluted	Good
Lactobacillus plantarum	8014	30-300	Good

Directions for Preparation from Dehydrated Product

BBL™ Orange Serum Agar

1. Suspend 45 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense in quantities under 50 mL and autoclave at 121°C for 10 minutes. For larger quantities, increase the autoclave time. Avoid overheating with consequent darkening and poor solidification.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Orange Serum Broth Concentrate 10×

1. To prepare the single-strength medium, aseptically add 100 mL of Orange Serum Broth Concentrate 10× to 900 mL sterile purified water and mix thoroughly.
2. Aseptically dispense 10 mL amounts into sterile test tubes.

Procedure

BBL™ Orange Serum Agar

1. For the plate count method, prepare serial 10-fold dilutions of the test material.
2. Add 1 mL of test sample to a sterile Petri dish.
3. Add 18-20 mL of molten agar (cooled to 45-50°C) and swirl plate gently to mix well.
4. Allow to solidify before incubating at 30°C for 48 hours. Plates can be held up to 5 days.

Difco™ Orange Serum Broth Concentrate 10×

Orange Serum Broth Concentrate 10× diluted to single-strength is used for small samples to initiate growth.

Expected Results

BBL™ Orange Serum Agar

Record colony morphology for each type of growth.

Difco™ Orange Serum Broth Concentrate 10×

Turbidity indicates growth.

Limitations of the Procedure

1. Orange Serum Agar is not a differential medium. Perform microscopic examination and biochemical tests to identify isolates to genus and species if necessary.
2. If Orange Serum Agar is divided into aliquots and allowed to solidify, remelt only once. Repeated heating may produce a softer medium.

References

1. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
2. Hays. 1951. Proc. Fla. State Hortic. Soc. *54*:135.
3. Murdock, Folinazzo and Troy. 1952. Food Technol. *6*:181.
4. Stevens. 1954. Food Technol. *8*:88.
5. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

BBL™ Orange Serum Agar

COMPF

Cat. No. 211486 Dehydrated – 500 g

Mexico

Cat. No. 252613 Prepared Plates (60 × 15 mm-style) – Pkg. of 20*

Difco™ Orange Serum Broth Concentrate 10×

COMPF

Cat. No. 251810 Ampule – 6 x 100 mL*

**Store at 2-8°C.*