



## PRODUCT SPECIFICATION SHEET

### Orange Serum Agar (DM927)

#### Intended Use

Orange Serum Agar (DM927) is recommended for cultivation and enumeration of microorganisms associated with spoilage of citrus products, cultivation of *Lactobacilli* and other aciduric organisms and pathogenic fungi.

#### Product Summary and Explanation

Fruit juices are generally acidic, with pH values ranging from approximately 2.4 for lemon juice, to 4.2 for tomato juice. The low pH of these foods is selective for yeast, moulds and a few groups of aciduric bacteria. Citrus juices are susceptible to spoilage by lactic acid bacteria, primarily species of *Lactobacillus* and *Leuconostoc*, yeast and moulds. Microbial spoilage of these citrus fruit juices are most commonly due to aciduric microbes such as lactic acid bacteria and yeast.<sup>(1)</sup> The lactic acid bacteria include *Lactobacillus fermentum*, *L. plantarum*, and *Leuconostoc mesenteroides*. Orange Serum Agar is recommended by APHA<sup>(1)</sup> for cultivation of *Lactobacilli* and other aciduric organisms. 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.<sup>(2)</sup> 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.<sup>(3)</sup> Dehydrated agar medium containing orange serum was reported by Stevens.<sup>(4)</sup> Orange Serum Broth is used to initiate growth of saprophytic, pathogenic fungi in small samples.<sup>(5)</sup> 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.

#### Principles of the Procedure

Orange Serum Agar contains casein enzymic hydrolysate provides essential nitrogenous nutrients while dextrose serves as the fermentable carbohydrate and energy source. Yeast extract supplies B-complex vitamins, which stimulate growth. Orange serum provides an optimal environment for the recovery of acid tolerant microorganisms from citrus fruit products. Dipotassium phosphate buffers the medium.

#### Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	10.00
Yeast extract	3.00
Dextrose	4.00
Dipotassium phosphate	2.50
Orange serum (Solids from 200 ml)	9.00
Agar	17.00
Final pH: 5.5 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

#### Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

#### Directions

1. Suspend 45.5 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. AVOID OVERHEATING.
5. Mix well and pour into sterile Petri plates.



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### Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Medium to dark amber coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 4.55% Solution	pH : 5.5 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.7% agar gel

**Expected Cultural Response:** Cultural characteristics observed after an incubation at 35-37°C for 40-48 hours. (Fungal species are incubated at 25-30°C)

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Recovery
1.	<i>Aspergillus brasiliensis</i> ATCC 16404	50 - 100	good-luxuriant	--
2.	<i>Candida albicans</i> ATCC 10231	50 - 100	good-luxuriant	≥50%
3.	<i>Lactobacillus acidophilus</i> ATCC 4356	50 - 100	good-luxuriant	≥50%
4.	<i>Lactobacillus fermentum</i> ATCC 9338	50 - 100	good-luxuriant	≥50%
5.	<i>Leuconostoc mesenteroides</i> ATCC 12291	50 - 100	good-luxuriant	≥50%
6.	<i>Saccharomyces cerevisiae</i> ATCC 9763	50 - 100	good-luxuriant	≥50%

The organisms listed are the minimum that should be used for quality control testing.

### Test Procedure

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.

### Results

Record colony morphology for each type of growth. Refer to appropriate references and procedures for results.

### Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

### Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

### 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.
3. Consult appropriate texts for detailed information and recommended procedures.

### Packaging

Product Name : Orange Serum Agar

Product Code : DM927

Available Pack sizes : 500gm



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### 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 Hort. 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.



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