Technical Data Sheet

Orange Serum Agar

Ordering number: 1.46425.0006

Orange Serum Agar is used for the isolation, cultivation and bacterial count of acid-tolerant microorganisms that cause deterioration of fruit juices and fruit-juice concentrates, particularly those of citrus fruits. It is generally used in fruit-juice production and to confirm the presence of acid-tolerant microorganisms in food packaging materials.

The nutrient medium conforms to the recommendation of the institute for the food industry and packaging (1974).

Orange Serum Agar is also available as tubes with 18 ml filling volume (article number 146350).

Mode of Action

Casein peptone covers the general growth requirements for carbon and nitrogen compounds, yeast extract provides B-complex vitamins and trace elements. Glucose is also a source of carbon and energy. In combination with the low pH value of the medium, the additional acid formation from glucose favors the growth of yeasts, molds, Lactobacillus spp. and Leuconostoc spp.. These spoilage agents are particularly stimulated by the addition of orange extract (orange serum).

Typical Composition

Casein Peptone	10 g/l
Yeast Extract	3 g/l
Orange Extract	5 g/l
Glucose	4 g/l
K_2HPO_4	3 g/l
Agar	18 g/l

The appearance of the medium is clear and yellowish. The pH value is in the range of 5.3-5.7. The medium can be adjusted and/or supplemented according to the performance criteria required.

Application and Interpretation

The medium can be melted by placing in a boiling water bath as specified in ISO 11133. *Note: Avoid over heating the medium. Remove it from the boiling water bath once melted.* Transfer the molten medium in a thermostatically controlled water bath. Maintain temperature from 47°C to 50°C. It is recommended to use the medium as soon as possible.

For the bacterial count by the pour-plate method the agar is liquefied at 95 °C in a water bath (test tube 10 minutes, 200-ml bottles 30 minutes) and then cooled to 44-47 °C in a second water bath (time as

above). The complete process of liquefaction, cooling and tempering at 44-47 °C should not take longer than 4 hours. The liquid agar is then poured over the samples in Petri dishes and mixed by swirling it around.

The dishes can be incubated aerobically or under micro-aerophilic conditions with increased CO_2 content using Anaerocult® C (article number 116275) or Anaerocult® C mini (article number 113682) for 2 to 5 days at 25-30 °C.

The growth of fungi can be mostly suppressed under micro-aerophilic conditions and at the same time the growth of micro-aerophilic lactic-acid bacteria is promoted.

Because the Orange Serum Agar is only weakly selective, mature colonies must be identified.

The selective nutrient media Yeast Glucose Chloramphenicol (YGC) Agar (article number 146348) for fungi and MRS agar (article number 146717) for *Lactobacillus spp.* are suitable for further identification. Candida-Ident Agar and Cereus-Ident Agar (article number 146092) are available as chromogenic selective nutrient media.

Leuconostoc spp. are gram-positive and typically appear as pairs or chains under the microscope. They are immovable, indolyl and catalase-negative and form acids from glucose (for differentiation see, e.g. Garvie 1984).

Storage and Shelf Life

The product can be used for sampling until the expiry date if stored upright, protected from light and properly sealed at +2 °C to +25 °C.

The testing procedures as described on the CoA can be started up to the expiry date printed on the label.

Disposal

Please mind the respective regulations for the disposal of used culture medium (e.g. autoclave for 20 min at 121 °C, disinfect, incinerate etc.).

Quality Control

Control Strains	ATCC #	Inoculum CFU	Incubation	Expected Results
Saccharomyces cerevisiae	9763	~100	44-48 h at 35-37 °C	good growth; yellowish, medium-sized colonies
Candida albicans	10231	~100	44-48 h at 35-37 °C	good growth; whitish, small, dry colonies
Lactobacillus rhamnosus	7469	~100	44-48 h at 35-37 °C (micro-aerophilic)	good growth; whitish, small colonies

Please refer to the actual batch related Certificate of Analysis.

Literature

Garvie, E.I. (1984): Separation of species of the genus *Leuconostoc* and the differentiation of the leuconostocs from other lactic acid bacteria. Methods Microbiol. **16**: 147-178.

Hays, G.L. (1951): The isolation, cultivation and identification of organisms which have caused spoilage in frozen concentrated orange juice. Proc. Fla. State Hortic Soc. **54**: 135-137.

ISO 11133:2014: Microbiology of food and animal feed and water – Preparation, production, storage and performance testing of culture media

ISO 7218 AMD 1: 2013: Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations

Murdock, D.L., Folinazzo, J.F. and Troy, V.S. (1952): Evaluation of plating media for citrus concentrates. Food Technol. **6**: 181-185.

Working groups of the institute for food technology and packaging at the Technical University of Munich: publications for testing packaging, Publication 19, "Determining the bacterial count, the number of fungi and yeasts and the number of coliform microorganisms in bottles and similar narrow-necked containers". Verpackgs. Rdsch. **25**; Techn.-wiss. Beilage 569-575 (1974) und Milchwiss. **29**: 602-606 (1974).

Ordering Information

Product	Cat. No.	Pack size
Orange Serum Agar	1.46425.0006	6 x 200 ml bottles
Orange Serum Agar	1.46350.0100	100 x 18 ml tubes
Yeast Glucose Chloramphenicol (YGC) Agar	1.46348.0020	20 x 90 mm plates
ReadyPlate™ MRS (de Man, Rogosa and Sharpe) Medium acc. ISO 15214	1.46717.0020	20 x 90 mm plates
ReadyTube™ 200 MRS (de Man, Rogosa and Sharpe) Medium acc. ISO 15214	1.46364.0006	6 x 200ml
Cereus-Ident-Agar	1.46092.0020	20 x 90 mm plates
Anaerocult® A (anaerobic jar)	1.13829.0001	10 x 1 pcs
Anaerocult® A mini (anaerobic jar)	1.01611.0001	25 x 1 set
Anaerobic jar 2.5L volume	1.16387.0001	1 unit
Anaerocult® C	1.16275.0001	25 x 1 set
Anaerocult® C mini	1.13682.0001	25 x 1 set
Anaerocult® P	1.13807.0001	25 x 1 set
Anaerotest®	1.15112.0001	50 strips

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