

Reference : 01-698 Product : ORANGE SERUM AGAR

## Also known as OSA

# Specification

Solid medium for the culture of aciduric organisms especially those associated with the spoilage of citrus products and their derivatives.

# Formula \* in g/L

Tryptone	10.00
Yeast extract	3.00
Orange serum	5.00
Dextrose	4.00
Dipotassium phosphate	3.00
Agar	17.00

#### Final pH 5.5 ±0.2 at 25 °C

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

#### Directions

Suspend 42 g of powder in 1 L of distilled water and boil to dissolve the agar. Distribute in suitable containers and autoclave for 15 minutes at 121°C. Avoid unnecessary overheating so as to minimise the darkening (caramelisation) and loss of gelification of the medium. It is recommended that the medium be used immediately after preparation.

#### Description

Orange Serum Agar was developed in the 1950's by Hays and coworkers for the detection, enumeration and isolation of spoilage microoganisms in fruit juices and products derived from citrus. Products with a low pH have microbial growth restricted to that of aciduric microorganisms. In a later study it was shown that Orange Serum Agar pH 5.4 was the most suitable medium for the isolation of lactic acid bacteria, especially (*Lactobacillus* and *Leuconostoc*) and yeasts that produce (buttermilk off-odour) in citrus fruits.

Orange Serum Agar is not a differential Agar but a culture medium in which the orange extract provides a favourable acidic environment in which aciduric microorganisms can be recovered including those damaged by food processing. Tryptone provides the main source of carbon and nitrogen, providing optimal growth conditions. Yeast Extract supplies Group B complex vitamins that stimulate growth and the phosphate provides an osmotic buffer for cell survival. Dextrose is a supplementary source of carbon and the agar is a solidifying agent.

## Technique

The International Fruchtsaft-Union (IFU) recommends the use of Sérum d'orange Agar in several standardized methods, using the plate count method:

- 1. Prepare 10-fold serial dilutions of the sample using a suitable diluent such as buffered Peptone Water.
- 2. Distribute aliquots of 1 ml of the diluted sample in sterile Petri dishes.
- 3. Add 20 ml of molten sterile medium cooled to 45 °C, gently swirl the dish to mix the sample and medium properly.
- 4. Allow it to solidify and incubate à 30 ±1 °C for 48 hours before enumeration. If there is no growth extend the incubation to 5 days, reading daily before giving a negative result.

Generally the colonies of yeasts and moulds are distinguished by their morphology but those of aciduric bacteria need to be Gram stained and examined microscopically to be appropriately categorised.

## Quality control

Incubation temperature:	30 ± 1 °C MicroAE	Incubation time:	48 h-5 days	
Inoculum: Practical range 100 ±20 CFU. min. 50 CFU (productivity), according to ISO 11133:2014/Amd 1:2018.				
Microorganism	Growth		marks	
Lactobacillus fermentum ATCC <sup>®</sup> 9338	Productivity >	• 0.50 -		
Saccharomyces cerevisiae ATCC <sup>®</sup> 97	63 Productivity >	• 0.50 -		
Aspergillus brasiliensis ATCC <sup>®</sup> 16404	Productivity >	> 0.50 Aer	obiosis	



# References

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## Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).