Reference: 450731ZA **Technical Data Sheet**

Product: # Orange Serum Agar

Specification

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

Presentation

	Packaging Details	Shelf Life	Storage
10 Prepared bottles Bottle 250 ml with: 200 ± 5 ml	1 box with 10 bottles 250 ml. Injectable cap: Plastic screw inner cap. The use of syringes needles with a diameter greater than 0.8 mm is not recommended	18 months	8-25 ºC

Composition

Composition (g/l):

Orange Serum	5.00
Yeast extract	3.00
Tryptone	10.0
Dextrose	4.00
Dipotassium phosphate	3.00
Agar	17.0

Description /Technique

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 Orange serum agar in several standardised methods, using the plate count method:
1. Prepare serial 10-fold 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 45ºC, gently swirl the dish to mix the sample and medium properly.
4. Allow it to solidify and incubate at a 30 ± 1ºC 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.

Note: The solid mediums can be melted in different ways: autoclave, bath and, if the customer considers appropriate, also the microwave. Whenever the microwave option is chosen, it is necessary to take certain safety measures to avoid breaking of the containers, such as loosening the screw cap and putting the bottle or tube in a water bath in the microwave. The fusion temperature and time will depend on the shape of the container, the volume of medium and the heat source. Avoid overheating as both the heating periods.

Quality control

Physical/Chemical control

Color : Pale yellow pH: 5.5 ± 0.2 at 25ºC

Microbiological control

Melt Medium - Prepare Plates - Spiral Spreading: Practical range 100 ± 20 CFU. min. 50 CFU (productivity)

Microbiological control according to ISO 11133:2014/A1:2018.

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosis. Incubation at 30 ± 1 ºC Reading at 48 h - 5 days

Microorganism	Growth
S. cerevisiae ATCC® 9763, WDCM 00058	Good (≥50 %)
Lactobacillus fermentum ATCC® 9338	Good (≥50 %)
Aspergillus niger ATCC® 16404, WDCM 00053	Good (≥50 %)
Lactobacillus plantarum ATCC® 8014	Good (≥50 %)
Leuconostoc mesenteroides ATCC® 9135, WDCM 00108	Good (≥50 %)

Sterility Control

Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

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