APT AGAR

For the cultivation and enumeration of heterofermentative lactobacilli

Typical formula (g/l)

Tryptone	12.50
Yeast Extract	7.50
Glucose	10.00
Sodium Citrate	5.00
Sodium Chloride	5.00
Dipotassium Hydrogen Phosphate	5.00
Manganous Chloride	0.14
Magnesium Sulphate	0.80
Ferrous Sulphate	0.04
Sorbitan Monoleate	0.20
Agar	15.00
Thiamine HCl	0.10 mg

APT BROTH

For the cultivation and enumeration of heterofermentative lactobacilli

Typical formula (g/l)

Tryptone	12.50
Yeast Extract	7.50
Glucose	10.00
Sodium Citrate	5.00
Sodium Chloride	5.00
Dipotassium Hydrogen Phosphate	5.00
Manganous Chloride	0.14
Magnesium Sulphate	0.80
Ferrous Sulphate	0.04
Sorbitan Monoleate	0.20
Thiamine HCl	0.10 mg

Directions

Suspend 46.2 g of APT Broth or 61.2 of APT Agar in 1000 ml of cold distilled water; heat to boiling, distribute and sterilise by autoclaving at 121°C for 15 minutes. Do not overheat.
Final pH 6.7 ± 0.2

Description

Evans and Niven studied APT Agar and APT Broth for the cultivation of heterofermentative lactobacilli that produce greening of cured meat products. These media are also used for the conservation and preparation of the *Lactobacillus viridescens* ATCC 12706 strain, which is the test organism in the microbiological assay of thiamine according to the method described by Deibel et al. A.P.H.A. recommends the use of APT Agar for the detection of lactobacilli in foodstuffs.

Technique

The technique suggested is the standard plate count; the details change according to the material to be tested.

Material to be tested	Diluent	Incubation
Sauerkraut	distilled water	32°C for 3 days
Fruit juices	distilled water	32°C for 6 days
Salted canned meat	phosphate buffer	21°C for 6 days

For the detection of H_2O_2 producing strains, APT Agar may be prepared with MnO_2: suspend 20 g of MnO_2 in 200 ml of APT Broth, distribute 10ml in tubes and sterilise by autoclaving at 121°C for 15 minutes. To 100ml of APT Agar add 10ml of MnO_2 suspension. Prepare the plates with a 15ml base layer of APT Agar without MnO_2, leave the medium to solidify then add 15 ml of a surface layer of APT Agar with MnO_2. Inoculate the sample and the sample dilutions onto the surface of the medium. H_2O_2 producing lactobacilli grow with colonies surrounded by a transparent halo.
As these media are non-selective and permit the growth of contaminants, the presumptive diagnosis of the presence of lactobacilli should be confirmed by microscopic and biochemical examinations.
APHA moreover, recommends an artificial pollution test to confirm the diagnosis of bacterial greening of canned meats. Transfer a few colonies from the APT Agar plates to APT Broth tubes and incubate at 32°C for 24 hrs. Prepare a moist sterile chamber (Petri dish with filter paper imbued with sterile water) and put a slice of the test material in this chamber under aseptic conditions. Inoculate the surface with a loopful of Broth culture in APT Broth; incubate at 32°C for 24 hours and observe whether the meat has greened. If it occurs and if an un-inoculated control specimen is found to be unchanged, the diagnosis is confirmed. The presence of greening due to exceeding nitrites is to be distinguished from the bacterial greening by carrying out identification tests and assays of nitrites with the standard reagents.

User quality assurance (APT Agar + MnO_2 : 30°C-5 days)
Productivity control
L.viridescens ATCC 12706: growth, colonies with transparent halo
L.brevis ATCC 14869: growth, colonies with transparent halo
L.sakie ATCC 215521: growth, colonies without transparent halo
L.mesenteroides DSM 20241: growth, colonies without transparent halo
P.damnosus ATCC 29358: growth, colonies without transparent halo

Storage
Dehydrated media: 10-30°C
User prepared plates and tubes: up to 7 days at 2-8°C

References
• APHA (1966) - Recommended Methods for the Microbiological Examination of Foods. 2nd. edition.
• D'Aubert S. (1963) Ann. Microbiol., **8**, 189
• Deibel, RH., Evans, J.B. & Niven, C.P. Jr. (1957) - J. Bact., **74**, 818-821.
• Niven, C.F. Jr. & Evans, J.B. (1957) - J. Bact., **73**, 758-759.
• Niven, C.F. Jr. Castellani, A.G. & Allanson V. (1969) - J. Bact., **58**, 633-641.
• Shipp, H.L. (1964) Technical Circular n° 266. British Food Manufacturing Industries Research Association, Leatherhead, U.K.

Packaging

4010851	**APT Agar,**	**100 g (1.6 l)**
4010852	**APT Agar,**	**500 g (8.1 l)**
4010901	**APT Broth,**	**100 g (2.1 l)**
4010902	**APT Broth,**	**500 g (10.8 l)**