

Specification

Medium for aerobic plate counts by the surface inoculation method (standard Plate Count Agar) according to ISO 4833, 8552 & 17410 Standards and IFU No. 6.

Presentation

	Packaging Details	Shelf Life	Storage
10 Prepared bottle Bottles 500 ml with: 450 ± 5 ml	1 box with 10 bottles 500 ml. Plastic screw inner cap.	16 months	8-25 °C

Composition

Composition (g/l):	
Casein peptone.....	5.00
Yeast extract.....	2.50
Dextrose.....	1.00
Agar.....	15.0

Description /Technique

Description

The Plate Count Agar formulation is according to that of Buchbinder et al. as recommended in their study of media for the plate count of microorganisms.

The original formulation of the standardized agar for dairy microbiology has been modified in order to avoid the addition of milk. This new composition allows the growth of most microorganisms without any further additions.

This medium's formulation is equivalent to that described by the 'Standard Methods for the Examination of Dairy products', the USP's 'Tryptone Glucose Yeast Agar', the 'Deutsche Landwirtschaft' and to the APHA and AOAC's Plate Count Agar. This is the medium of choice for the plate count of any type of sample

Technique

To use, the contents of the bottle should be poured into plates. The melting of the culture medium should be carried out according to the manufacturer's instructions, either in a water bath or microwave oven. Never apply direct heat to melt a medium. The melting temperatures and times depend on the shape of the container, the volume of medium and the heat source. Before melting any medium loosen the screwcap of the container to avoid breaking the container. The medium should be melted only once and used. Media with agar should not be melted repeatedly as their characteristics change with each remelting. Overheating should be avoided as much as prolonged heating, especially with regard to media with an acidic or alkaline pH. Once melted pour the plates using aseptic techniques. To inoculate, follow standard laboratory methods or the applicable norms. Spiral plate method, streak plating, econometric methods, dilution banks, spread plating etc.

Prepare ten fold serial dilutions of the sample and take 1 mL aliquots in duplicate from each dilution and put them into sterile Petri plates. Pour 20 mL approx. of sterile cooled medium (around 45°C) in each of the plates. Mix gently by swirling the plate in the form of a figure 8. Leave the plates undisturbed to solidify and incubate in an inverted position. The incubation time and temperature depend on the type of microorganism under study. For a general aerobic count, incubate for 3 days at 30°C. Taking readings after 24, 48 and 72 hours.

The plate count method proposed by the APHA consists of pouring the molten agar at 50°C on plates containing the diluted samples (pour plate technique). The final count is carried out after 48 hours of incubation at 32 -35°C.

For microorganisms with other temperature requirements, the following incubations have been suggested: 2 days at 32-35°C, 2-3 days at 45°C, 2 days at 55°C, 3-5 days at 20°C, 7-10 days at 5-7°C.

Sample dilutions are prepared with 1/4 Ringer's solution, Buffered Peptone Water or Maximum Recovery Diluent depending on their nature.

The poured plate count method is preferred to the spread plate technique, since it gives higher counts.

Nevertheless, the latter facilitates isolation and reseeded of the colonies.

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 : Yellowish pH: 7.0 ± 0.2 at 25°C

Microbiological control

Melting - pour plates - inoculation Practical range 100 ± 20 CFU. min. 50 CFU (productivity) / 10⁴-10⁶ CFU (selectivity)

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 °C, reading at 24-48-72 h

Microorganism

Bacillus subtilis ATCC® 6633, WDCM 00003

Escherichia coli ATCC® 8739, WDCM 00012

L. monocytogenes ATCC® 35152, WDCM 00109

Stph. aureus ATCC® 25923, WDCM 00034

Growth

Good (≥70%)

Good (≥70%)

Good (≥70%)

Good (≥70%)

Sterility Control

Incubation 48 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

Bibliography

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
- BUCHBINDER, L., Y. BARIS & L. GOLDSTEIN (1953) Further studies on new milk-free media for the standard plate count of dairy products. Am. J. Public Health 43:869-872.
- CLESCERI, L.S., A.E.GREENBERG and A.D. EATON (1998) Standard Methods for the Examination of Water and Wastewater. 20th ed., APHA, AWWA, WPCF. Washington.
- DIN 10192 (1971) Prüfungsbestimmungen für Milch und Milcherzeugnisse. Deutsche Landwirtschaft, Fachbereich Ernährung.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed., APHA, Washington.
- FIL/IDF Standards 3 (1958), 100, 101 (1981), 109 (1982) and 132 (2004).
- HORWITZ, W. (2000) Official Methods of Analysis. AOAC International. Gaithersburg.
- IFU Method No 6 (1996) Mesophilic, thermophilic and thermophilic bacteria: Spores Count. D-1 Mesophilic Aerobic Sporeforming bacteria: Spores count.
- ISO 4833 (2003) Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony count technique at 30°C.
- ISO 8552 (2004) Milk - Estimation of psychrotrophic microorganisms. Colony count technique at 21°C (Rapid method).
- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- ISO 17410 (2001) Horizontal method for the enumeration of psychrotrophic microorganisms.
- MARSHALL, R.T. (1992) Standard Methods for the Examination of Dairy Products. 16th ed. APHA. Washington.
- PASCUAL ANDERSON. M^a.R^o. (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.