

PLATE COUNT AGAR WITH SKIM MILK

Dehydrated culture medium

1 - INTENDED USE

For microbial plate count in milk and dairy products.

2 – COMPOSITION*

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER)

Tryptone	5.0 g
Yeast extract	2.5 g
Glucose	1.0 g
Skim milk	1.0 g
Agar	15.0 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Plate Count Agar supplemented with skim milk is recommended by ISO Standards¹⁻³ for the enumeration of mesophilic or psychrotrophic microorganisms in milk and dairy products.

This test is based on the assumption that each viable cell, pair of cells, or small cluster of cells will form a visible colony, called a colony-forming unit (CFU), when mixed with the growth medium.⁴

Enumeration of microorganisms requires diluting samples to achieve a population that is countable by the chosen method.

Several techniques have been described and are available for aerobic plate counts: pour plate technique, surface spread plate method, membrane filtration, spiral plate method, calibrated loop method, drop plate method.⁴ The choice of the most appropriate method must take into account the requirements of the regulatory authorities, the type of sample to be analysed, the expected microorganisms and level of contamination.

The International Standard ISO 4833-1 specifies a pour plate method for the enumeration of mesophilic organisms and is applicable to products that require a reliable count when a low limit of detection is specified or to products expected to contain spreading colonies.¹

ISO 4833-2 specifies a surface plating technique applicable to products containing heat sensitive organisms or obligately aerobic bacteria.²

ISO 17410 describes a surface plating method for the enumeration of psychrotrophic microorganisms with incubation at 6.5°C.³

The formulation of Plate Count Agar with Skim Milk complies with ISO Standards.¹⁻³ Tryptone provides nitrogen, carbon, minerals and amino acids for the microbial growth. Yeast extract is a source of vitamins, particularly of the B-group. Glucose is a source of carbon and energy. The skimmed milk included in the formulation is tested free of antibiotics.

4 - DIRECTIONS FOR DEHYDRATED MEDIUM

Suspend 24.5 g in 1000 mL of cold purified water. Heat to boiling with frequent agitation to dissolve completely and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C, mix well and distribute into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared medium appearance	pale beige, clear or slightly opalescent
Final pH at 20-25 °C	7.0 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Plate Count Agar with Skim Milk	Dehydrated medium	4019182	500 g (20.4 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile pipettes and spreaders, incubator and laboratory equipment as required, Erlenmeyer flasks, sterile Petri dishes, ancillary culture media and reagents.

8 – SPECIMENS

Milk and dairy products. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards.¹⁻³

9 - TEST PROCEDURE

Colony count by the pour plate technique.¹

- Using a sterile pipette, dispense 1 mL of the liquid test sample, or 1 mL of an initial suspension in the case of other products, into an empty Petri dish and mix with the molten medium pre-cooled to 44-46°C.
- Prepare the other plates under the same conditions using decimal dilutions of the test sample or of the initial suspension.
- Incubate the plates under aerobic conditions at 30 °C for 72 h.

Colony count by the surface plating technique.^{2,3}

- Dry the prepared plates before the use.
- Using a sterile pipette, transfer 0.1 mL of the test sample, if the product is liquid, or of the initial suspension in the case of other products, to the centre of a Plate Count Agar with Skim Milk plate.
- Carefully spread the inoculum uniformly and as quickly as possible over the surface of the agar plate, without touching the sides of the dish with the spreader.
- Leave the plates with the lids on for about 15 min at ambient temperature for the inoculum to be absorbed into the agar.
- Incubate the plates under aerobic conditions at 30 °C for 72 h for the enumeration of mesophilic organisms or at 6.5°C for 10 days for the enumeration of psychrotrophic microorganisms.

Consult the appropriate International Standard for the details of the procedures.¹⁻³





10 - READING AND INTERPRETATION

After incubation, count all colonies obtained in the plates containing fewer than 300 colonies and calculate the number of microorganisms per gram or per millilitre of the test sample.

Follow recommended procedures for the counting of colonies and the reporting of results.¹⁻³

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS
<i>E. coli</i> ATCC 8739	30°C/72H-A	good growth
<i>S. aureus</i> ATCC 6538	30°C/72H-A	good growth
<i>B. subtilis</i> ATCC 6633	30°C/72H-A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 – PERFORMANCE CHARACTERISTICS

Prior to release for sale, a representative samples of all lots of dehydrated Plate Count Agar with Skim Milk (Test batch:TB) is tested for productivity by comparing the results with Tryptic Soy Agar (TSA).

The productivity is tested by a quantitative pour plate method with the following strains *E. coli* ATCC 8739, *S. aureus* ATCC 6538, *B. subtilis* ATCC 6633, and *L. casei* ATCC 7469. The plates are inoculated by pour plate technique with decimal dilutions in saline of a colonies' suspension and incubated at 30°C for 72 hours. The colonies are enumerated on both media and the productivity ratio (Pr: CFU_{TB}/CFU_{TSA}) is calculated. If Pr is ≥ 0.7 the results are considered acceptable and conform to the specifications.

13-LIMITATIONS OF THE METHODS

- A delay of more than 10 minutes between sample dispensing into Petri dishes and agar addition can result in lower counts.^{4,5}
- A potential source of error in plate count can result from the stack-pouring Petri dishes: in a stack of 3 plates, the middle and the top plates took too longer to cool, thereby resulting in lower counts.^{4,6}
- Increasing the holding time of the dilutions in the diluent leads to higher count.^{4,7}
- The Aerobic Plate Count does not differentiate between different type of bacteria. Alteration in incubation time and temperature and the type of atmosphere will change the types of organisms that will grow and thus be counted.⁴

14 - PRECAUTIONS AND WARNINGS

- This product is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/tubes/bottles) and the applied storage conditions (temperature and packaging). According to ISO Standards, self-prepared flasks can be stored at +2 °C to +8 °C for up to 3 months and the self-prepared plates can be stored at +2 °C to +8 °C for up to 4 weeks.^{1,3}















16 - REFERENCES

1. ISO 4833-1:2013. Microbiology of the food chain – Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30 °C by the pour plate technique.
2. ISO 4833-2:2013. Microbiology of the food chain – Horizontal method for the enumeration of microorganisms - Part 2: Colony count at 30 °C by the surface plating technique.
3. ISO 17410:2019. Microbiology of the food chain — Horizontal method for the enumeration of psychrotrophic microorganisms
4. American Public Health Association. Compendium of Methods for the Microbiological Examination of Foods, 5th ed. 2015. APHA, Washington, DC.
5. Berry JM, McNeill DA, Witter LD. Effect of delay in pour plating on bacterial counts. J Dairy Sci 1969; 52:1456-1457
6. Koburger JA. Stack pouring of Petri plates: a potential source of error. J Food Prot. 1980; 43:561-562.
7. Huhtanen CN Brazis AR, Arledge WL et al. Effects of time of holding dilutions on counts of bacteria from raw milk. J Milk Food Technol. 1972; 35:126-130.

TABLE OF APPLICABLE SYMBOLS

 or  Catalogue number	 Batch code	 Manufacturer	 Store in a dry place	 Use by
 Temperature limitation	 Contents sufficient for <n> tests	 Consult Instructions for Use	 Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/11

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

