

MILK PLATE COUNT AGAR (7703)

Intended Use

Milk Plate Count Agar is used for the isolation and enumeration of microorganisms in milk and dairy products.

Product Summary and Explanation

Liquid milk and other dairy products are highly perishable and can spoil in a few days. Contamination of raw milk can arise from several sources including, soiled udders, inadequately cleaned milking equipment, and poor handling and processing of samples. Prolonged or improper holding of dairy products may permit microbial contamination to increase. Bovine mastitis may cause contamination with *Staphylococcus aureus*, *Streptococcus agalactiae*, *E. coli* and other microorganisms. Poor cleaning of the milking equipment may cause contamination with streptococci, coliforms, or heat resistant *Bacillus* spp. Spoilage of pasteurized or raw milk by proteolytic psychrotrophic bacteria can occur on prolonged storage below 7°C

Milk Plate Count Agar is equivalent to the medium recommended by the British Standard Institution¹, International Dairy Federation², and International Organization for Standardization³ for the enumeration of microorganisms in liquid milk, ice cream, dried milk, and whey.

Principles of the Procedure

The nitrogen and essential vitamins are provided by Tryptone and Yeast Extract. Glucose is the carbon energy source. Antibiotic Free Skim Milk is a source of casein. Proteolytic bacteria will be surrounded by a clear zone from the conversion of casein into soluble nitrogenous compounds.¹ Agar is the solidifying agent.

Formula / Liter

| | |
|---------------------------------|-------|
| Tryptone | 5 g |
| Yeast Extract..... | 2.5 g |
| Glucose | 1 g |
| Antibiotic Free Skim Milk | 1 g |
| Agar | 10 g |

Final pH: 6.9 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.

Directions

1. Dissolve 19.5 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is trace to slightly hazy and light beige to medium amber.

Expected Cultural Response: Cultural response on Milk Plate Count Agar was performed with raw milk dilutions. Raw milk dilutions were prepared and tested following the standardized test method as outlined in Milk Plate Agar for the Microbiological Examination of Dairy Products,⁴ incubated at $32 \pm 1^\circ\text{C}$, and examined for growth at 48 hours.

| Test Sample | Expected Results |
|--------------------------|------------------|
| Unpasteurized (raw) milk | t-value < 2.70 |

Milk Plate Count Agar was also inoculated with the organisms listed below. Cultures were incubated aerobically at $35 \pm 2^\circ\text{C}$ and examined for growth at 18 – 24 hours.

| Microorganism | Approx. Inoculum (CFU) | Expected Growth |
|---|------------------------|-------------------|
| <i>Escherichia coli</i> ATCC® 25922 | 10 - 300 | Good to excellent |
| <i>Staphylococcus aureus</i> ATCC® 25923 | 10 - 300 | Good to excellent |
| <i>Staphylococcus epidermidis</i> ATCC® 12228 | 10 - 300 | Good to excellent |
| <i>Streptococcus pneumoniae</i> ATCC® 6305 | 10 - 300 | Good to excellent |
| <i>Streptococcus pyogenes</i> ATCC® 19615 | 10 - 300 | Good to excellent |

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Perform total counts using the pour plate method or spread plate procedure.

1. Prepare milk dilutions of 1:10, 1:100, 1:1000, in ¼ strength Ringer's solution. Use this inoculum within 15 minutes.
2. **Pour Plates:** Pipette 1 mL of each dilution into Petri dishes. Add 10 – 12 mL of Milk Plate Count Agar, cooled to 45°C . Mix thoroughly.
3. **Spread Plates:** Spread 1 mL of each milk dilution over the surface of each prepared and solidified Milk Plate Count Agar.
4. Incubate inoculated medium at $35 \pm 2^\circ\text{C}$ and examined for growth.

Results

Select plates containing 10 – 300 colonies. Results are expressed as colonies per mL of product tested.

Proteolytic psychrotrophic colonies may be enhanced by flooding the plates with a solution of 1% hydrochloric acid or 10% acetic acid. Pour off the excess acid solution and count the colonies surrounded by clear zones.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C . Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

| | | | |
|-----------------------|----------|-------|-------|
| Milk Plate Count Agar | Code No. | 7703A | 500 g |
| | | 7703B | 2 kg |
| | | 7703C | 10 kg |

References

1. **British Standards Institution.** BS4285 Sec. 1.2. 1984. Microbiological examination for dairy purposes. Diluents, media and apparatus and their preparation and sterilisation.
2. **International Dairy Federation.** 1020 Brussels, Belgium.
3. **International Organization for Standardization.** 1982. International Standard ISO / DIS 6610.
4. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.