# **Specification**

Solid medium for the plate count of milk and dairy products, according to DIN and FIL/IDF standards.

### Formula \* in q/L

Tryptone	5.00
Yeast extract	2.50
Skimmed milk	1.00
Dextrose	1.00
Agar	10.50

#### Final pH 7.0 ±0.2 at 25 °C

#### **Directions**

Suspend 20 g of powder in 1 L of distilled water and let it soak. Bring to the boil, constantly stirring. Distribute into suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

## Description

This medium, with added milk, is more nutrient rich than other standard media; however, the opalescence of the medium makes early observations sometimes difficult.

Due to its lower agar concentration, it may be used for the pour plate method or the spread plate method.

### **Technique**

Prepare 10-fold serial dilutions of the sample and take 1 ml in duplicate aliquots from each dilution and put them in sterile Petri dishes. Pour approx. 20 ml of sterile cooled medium (around 45 °C) in each of the plates. Mix gently by swirling the plate in 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 investigation. In general for an aerobic count, incubate for 3 days at 30 °C. Checking the plates at 24, 48 and 72 hours.

The plate count method proposed by the APHA consists of the pour plate method i.e. pouring the molten agar at 50 °C on plates containing the diluted samples. 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 30 ±1 °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 surface inoculation method, since it gives higher counts, although the latter facilitates isolation and reseeding of the colonies.

# **Quality control**

Incubation temperature:  $30^{\circ}\text{C} \pm 1.0$  Incubation time:  $72 \pm 3 \text{ h}$ 

Inoculum: Practical range 100±20 CFU. min. 50 CFU (productivity), according to ISO 11133:2014/Amd 1:2018. Spiral Plate Method.

Microorganism	Growth	Remarks
Escherichia coli ATCC® 25922	Productivity > 0.70	-
Bacillus subtilis ATCC® 6633	Productivity > 0.70	-
Staphylococcus aureus ATCC® 25923	Productivity > 0.70	-
Escherichia coli ATCC® 8739	Productivity > 0.70	-

<sup>\*</sup> Adjusted and /or supplemented as required to meet performance criteria

#### References

- · 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) StandardMethods for the Examination of Water and Wastewater. 20th ed., APHA, AWWA & WPCF. Washington.
- · DIN 10192 (1971) Prüfungesbestimmungen für Milch und Milcherzeugnisse. Deutsche Landwirtsachft, Fachbereit und Ernahrung.
- · 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 of the A.O.A.C. AOAC International. Gaithersburg. Va.
- IFU Method No 6 (1996) Mesophilic, thermoduric 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 techniqueat 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. Ma.Ro. (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.

#### **Storage**

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).