

17186 PM Indicator Agar (Penicillin in Milk Indicator Agar) NutriSelect® Plus

For rapid detection of trace amounts of Penicillin in milk using *Bacillus stearothermophilus*.

Composition:

Ingredients	Grams/Litre
Peptic digest of animal tissue	5.0
Casein enzymic hydrolysate	1.7
Papaic digest of soyabean meal	0.3
Beef extract	3.0
Dextrose	5.25
Sodium chloride	0.5
Dipotassium phosphate	0.25
Polysorbate	80.0
Bromocresol Purple	0.06
Agar	15.0

Final pH 7.8 +/- 0.2 at 25°C

Store granulated media below 30°C in tightly closed container and the prepared medium at 2- 8°C. Use before expiry date on the label.

Appearance(color): Faint Blue to Blue and Faint Green to Green, free flowing powder
 Gel strength: Firm, comparable with 1.5% agar gel
 Color and Clarity: Purple coloured clear to slightly opalescent gel forms in Petri plates.

Directions:

Suspend 3.2 g in 100 ml distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C

Principle and Interpretation:

PM Indicator Agar is used for rapid detection of trace amounts of penicillin in milk where AOAC has recommended *Bacillus stearothermophilus* qualitative discs (1). PM Indicator agar is designed according to the formula published by the 18th Annual Meeting of the National Mastitis Council (2). This method is a modification of the method approved by the International Dairy Federation for the qualitative detection of penicillin in milk (3). Originally a medium called Reductase Medium was suggested by Reid and Brewer for detecting penicillin in milk by using *Bacillus subtilis* (4). The present medium is better, faster, and more reliable than that of Reid and Brewer (This medium is designed to support and demonstrate the growth and acid formation by *B. stearothermophilus*, which is sensitive to penicillin and b-lactam residues). To demonstrate the presence of traces of penicillin in milk; the qualitative disc method is found to be more suitable which is also recommended by AOAC.

Inoculate the medium with 1 ml of uniformly dispersed *B. stearothermophilus* suspension prepared as per AOAC (1). Prepare dilutions of standard penicillin G (positive control) to give concentrations in the range of 0.005 to 0.1 units/ml. Use a suspension of inhibitor free non-fat dry milk for negative control.

For Screening Assay: Place a blank control disc for adding the sample, a negative control disc (inhibitor free non-fat dry milk) and a positive control disc on agar surface. Invert the plate and incubate till 17-20 mm zones of inhibition are obtained around positive control disc



Interpretation of Screening Assay

Test Sample Disc	Positive control	Negative control	Interpretation
No zone	Clear zone (17 - 20 mm)	No zone	No significant amounts of inhibiting substances.
Clear zone (< 14 mm)	Clear zone (17 - 20 mm)	No zone	significant amounts of inhibitory substances are not present
Clear zone (> 14 mm)	Clear zone (17 - 20 mm)	No zone	an inhibitor is present in test sample. Perform CONFIRMING ASSAY to determine if inhibitor is a beta-lactam residue.
Any reaction	Any reaction	Any zone	error in the test system
Any reaction	No zone	Any reaction	Determine source of error.

For Confirming Assay: Inactivate milk sample by heating at 82°C for 2 minutes. Cool promptly to room temperature. Invert the plate and incubate as in screening assay.

Interpretation of Confirming Assay

Inactivated Test Sample Disc	Positive control	Penicillinase / Inactivated Milk Sample Disc	Interpretation
Clear zone (> 14 mm)	Clear zone (17 - 20 mm)	No zone	indicating the presence of beta- lactam residues
Clear zone (> 14 mm)	Clear zone (17 - 20 mm)	Clear zone same size as test sample	presence of inhibitor(s) other than beta-lactam residues.
Clear zone (> 14 mm)	Clear zone (17 - 20 mm)	Clear zone substantially smaller than 14 mm	presence of beta-lactam residues as well as other inhibitors

Cultural characteristics observed after an incubation in following parameters.

Organisms (ATCC)	Growth	Incubation temperature	Time
<i>Bacillus stearothermophilus</i> (7953/-)	++	55 ± 2°C	3-4 hours
<i>Bacillus stearothermophilus</i> (7953/-)	+++	64 ± 2°C	3-4 hours

References:

1. Williams, (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C
2. Publ. of the 18th Annual Meeting of Natl. Mastitis Council, Inc.
3. International Dairy Federation, 1970, International Dairy Federation, Brussels, Belgium.
4. Reid R. D. and Brewer J. H., 1946, J. Bacteriol., 52: 251.

Precautions and Disclaimer

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