



Dichloran Medium Base with Rose Bengal

M1000

Dichloran Medium Base with Rose Bengal is used for selective isolation and enumeration of fungi-yeasts and moulds of significance in food spoilage.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Dextrose	10.000
Monopotassium phosphate	1.000
Magnesium sulphate	0.500
Rose bengal	0.025
Dichloran	0.002
Agar	15.000
Final pH (at 25°C)	5.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15.76 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add sterile reconstituted contents of 1 vial of Chloramphenicol Selective Supplement (FD033). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Dichloran Medium Base with Rose Bengal is formulated as described by King et al (1), which is a modification of Rose Bengal Chloramphenicol Agar (2). Dichloran Medium Base with Rose Bengal is used along with Rose Bengal Chloramphenicol Agar where it is necessary to enumerate yeasts in the presence of moulds.

Peptic digest of animal tissue provides nitrogen, vitamins and minerals. Dextrose is a carbohydrate source. Phosphate buffers the medium. Magnesium sulfate provides divalent cations and sulfate. Dichloran is an antifungal agent, added to the medium to reduce colony diameters of spreading fungi. Rose bengal exhibits an improved inhibitory activity at pH 5.6 and therefore the final pH of the medium is reduced to 5.6 for the inhibition of spreading fungi (1). The presence of rose bengal in the medium suppresses the growth of bacteria and restricts the size and height of colonies of the more rapidly growing moulds. The concentration of rose bengal is reduced for optimal performance with dichloran. Additionally, rose bengal is taken up by yeast and mold colonies, which allows these colonies to be easily recognized and enumerated.

Add 40 ml of food sample to 200 ml of 0.1% Peptone water (M028) and shake periodically for 30 minutes (3) or process in stomacher for 30 seconds (4). Inoculate 0.1 ml of this sample on Dichloran Medium Base with Rose Bengal. Report the number of colonies per gram of food.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Pink coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.15% w/v aqueous solution at 25°C. pH : 5.6±0.2

pH

5.40-5.80

Cultural Response

M1000: Cultural characteristics observed with added Chloramphenicol Selective Supplement (FD033), after an incubation at 25-30°C for upto 6 days.

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
<i>Bacillus subtilis</i> ATCC 6633	$\geq 10^3$	inhibited	0%
<i>Candida albicans</i> ATCC 10231	50-100	good-luxuriant	$\geq 50\%$
<i>Escherichia coli</i> ATCC 25922	$\geq 10^3$	inhibited	0%
<i>Mucor racemosus</i> ATCC 42647	50-100	good-luxuriant	
<i>Saccharomyces cerevisiae</i> ATCC 9763	50-100	good-luxuriant	$\geq 50\%$

Storage and Shelf Life

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

Reference

1. King D.A. Jr., Hocking A.D. and Pitt J.I., 1979, J. Appl. Environ. Microbiol., 37:959.
2. Jarvis B., 1973, J. Appl. Bact., 36:723.
3. Sharf J.M. (Ed.), 1966, American Public Health Association, 2nd ed., New York.
4. Sharp A.N. and Jackson A.K., 1972, J. Appl. Bact., 24:175.

Revision : 2 / 2015

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