Product :
DICHLORAN ROSE BENGAL CHLORAMPHENICOL AGAR (DRBC A.)

Also known as
DRBC Agar

Specification
Selective medium for the enumeration of moulds and yeasts in foodstuff, according to ISO standards.

Formula * in g/L
Mycological peptone..... 5.000
Dextrose..... 10.000
Potassium dihydrogen phosphate.....1.000
Magnesium sulphate..... 0.500
Dichloran..... 0.002
Rose bengal..... 0.025
Chloramphenicol..... 0.100
Agar.....15.000

Final pH 5.6 ±0.2 at 25 °C
* Adjusted and /or supplemented as required to meet performance criteria

Directions
Suspend 31,6 g of the powder in 1 L of distilled water and bring to the boil, continue boiling until completely dissolved. Distribute into suitable containers and sterilize in the autoclave at 121ºC for 15 minutes.

Description
Dichloran Rose Bengal Chloramphenicol (DRBC) Agar is a medium based on the Dichloran Rose Bengal Chlortetracycline medium developed by King *et al.* Cols (1979) and on the formulation of Rose Bengal Chlortetracycline medium of Jarvis (1973). The combination of Dichloran and Rose Bengal markedly restricts the size and height of mould colonies thus preventing overgrowth of luxuriant species and assisting accurate counting of colonies. The presence of Chloramphenicol and the low pH of 5.6 serve to prevent the growth of most bacteria. This medium supports good growth of yeasts and moulds and can be used to enumerate both toxigenic and non-toxigenic fungi but it is not diagnostic for detecting specific mycotoxin-producers.
In the current formulation the concentration of Rose Bengal is reduced to 25 µg/mL for optimal performance with Dichloran. Chlortetracycline is replaced by chloramphenicol as it is more stable and easier to handle. It is also preferred for use in the food and environmental sectors.
Rose Bengal is taken up by most yeasts and some moulds, which allows the easy recognition and enumeration of these colonies. Some times there can be a reduced recovery of certain yeasts due to increased activity of Rose Bengal at pH 5,6.

Technique
Using 0.1-0.2 ml of inoculum per 9 cm diameter plate, spread it over the whole surface of the plate. Incubate the plates upright à 25 °C for 5 days in the dark with examination for growth after 3, 4 and 5 days. Where identification is required prolong the incubation until characteristic colonies are formed. Colonies of yeast generally appear pink due the uptake of Rose Bengale.
Where separate counts of moulds and yeasts are required, identify by morphological appearance and perform microscopic examination of these two groups of microorganisms where necessary. Colonies of yeast and bacteria can be confused and microscopic examination should be carried out if unsure.

Cautions and Limitations
- Due to the selective properties of this medium and the type of specimen being cultured some strains of fungi can fail to grow or grow poorly.
- Similarly, some strains of bacteria may be encountered that are not inhibited or only partially inhibited.
- This medium is photo-sensitive. Do not expose to light since photo-degradation of Rose Bengale produces compounds toxic to fungi.
- The prepared medium have a short shelf-life and retain these à 4 ±2 °C in the dark.

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Quality control

Incubation temperature: 25 °C ± 1.0 **Incubation time:** ≤ 5 d

Inoculum: Practical range 100±20 CFU. min. 50 CFU (productivity)/ 10^4 -10□ CFU (selectivity), according to ISO 11133:2014/Amd 1:2018. Spiral Plate Method.

Microorganism	Growth	Remarks
Bacillus subtilis ATCC® 6633	Inhibited	-
Escherichia coli ATCC® 25922	Inhibited	-
Aspergillus niger ATCC® 16404	Productivity > 0.50	-
Saccharomyces cerevisiae ATCC® 9763	Productivity > 0.50	-
Candida albicans ATCC® 10231	Productivity > 0.50	-
Mucor racemosus ATCC® 42647	Productivity > 0.50	-

References

· ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Culture Media. CRC Press. Boca Raton. Fla. USA.

· BAYLIS, C.L. (2003) Manual of Microbiological Methods for the Food and Drinks Industry. CCFRA. Chipping Campden. Gloucestershire. UK.

· BEUCHAT, L.R. & M.A. COUSIN (2001) Yeasts and Molds. In Downes and Ito (ed.) Compendium of methods for the microbiological examination of foods. 4th ed. APHA. Washington. USA.

· CORRY, J.E.L., G.D.W. CURTIS & R.M. BAIRD (2003) Handbook of Culture Media for Food Microbiology. Elsevier Science. Amsterdam.

· ISO 21527-1 Standard (2008) Microbiology of food and animal feeding stuffs - Horizontal methods for the enumeration of yeast and moulds - Part1: Colony count technique in products with water activity greater than 0,95.

. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.

· JARVIS, B.(1973) Comparison of an improved Rose-Bengal-Chlortetracycline Agar with other media for the selective isolation and enumeration of moulds and yeasts in food. J. Appl. Bacteriol. 36:723-727.

· KING, D.A., A.D. HOCKING & J.J.PITT (1979) Dichloran-Rose Bengal medium for enumeration and isolation of molds from foods. Appl. Environm. Microbiol. 37:959-964.

Storage

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