# PRODUCT SPECIFICATION SHEET

## Rose Bengal Chloramphenicol Agar (DM346)

**Intended Use**
Rose Bengal Chloramphenicol Agar (DM346) is recommended for selective isolation and enumeration of yeast and mould from food and environmental material

**Product Summary and Explanation**
Various methods have been described for the selective isolation of fungi from environmental materials and foodstuffs containing mixed populations of fungi and bacteria. The use of media with an acid pH that selectively inhibits the growth of bacteria and thereby promotes the growth of fungi has been widely employed.[1-3] A number of investigators have reported, that, neutral pH media with antibiotics is advantageous for fungal growth compared to acidified media as the later may actually inhibit fungal growth,[4,5] fail to completely inhibit bacterial growth[5] and have little effect in restricting the size of mold colonies.[6] Smith and Dawson[7] used rose bengal in a neutral pH medium for the selective isolation of fungi from soil samples. Chloramphenicol, streptomycin, oxytetracycline and chlortetracycline have been used for the improved, selective isolation and enumeration of yeasts and molds from soil, sewage and foodstuffs.[4, 8-11] Rose Bengal Chloramphenicol Agar was formulated originally by Jarvis[11] and further modified by Overcast and Weakley.[10] Chloramphenicol is recommended because of its heat stability and broad antibacterial spectrum.

**Principles of the Procedure**
Rose Bengal Agar Base contains mycological peptone which provides the carbon and nitrogen sources required for good growth of a wide variety of organisms. Dextrose is an energy source. Monopotassium phosphate provides buffering capability. Magnesium sulphate provides necessary trace elements. Rose bengal is a selective agent that inhibits bacterial growth and restricts the size and height of colonies of the more rapidly growing molds. The restriction in growth of molds aids in the isolation of slow-growing fungi by preventing overgrowth by more rapidly growing species. Rose bengal is taken up by yeast and mold colonies, thereby facilitating their recognition and enumeration. Chloramphenicol has inhibitory action on gram-negative bacteria.

**Formula / Liter**

| Ingredients | Gms / Liter |
|---|---|
| Mycological peptone | 5.00 |
| Dextrose | 10.00 |
| Monopotassium phosphate | 1.00 |
| Magnesium sulphate | 0.5 |
| Rose Bengal | 0.05 |
| Chloramphenicol | 0.1 |
| Agar | 15.50 |
| Final pH: 7.2 ± 0.2 at 25°C | |
| Formula may be adjusted and/or supplemented as required to meet performance specifications | |

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**Precautions**

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

**Directions**

1. Suspend 32.15 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Mix well and pour into sterile petri plates.

**Quality Control Specifications**

| | |
|---|---|
| **Dehydrated Appearance** | Light yellow to pink homogeneous free flowing powder |
| **Prepared Medium** | Deep pink coloured clear to slightly opalescent gel forms in Petri plates. |
| **Reaction of 3.22% Solution** | pH : 7.2 ± 0.2 at 25°C |
| **Gel Strength** | Semisolid, comparable with 1.55% Agar gel |

**Expected Cultural Response:** Cultural characteristics observed after an incubation at 25-30°C for 5 days.

| Sr. No. | Organisms | Results to be achieved |
|---|---|---|
| | | Growth |
| 1. | *Aspergillus brasiliensis ATCC 16404* | good-luxuriant |
| 2. | *Bacillus subtilis ATCC 6633* | inhibited |
| 3. | *Cladosporium cladosporioidesATCC 11278* | good-luxuriant |
| 4. | *Escherichia coli ATCC 25922* | inhibited |
| 5. | *Enterococcus faecalis ATCC 29212* | inhibited |
| 6. | *Mucor racemosus ATCC 42647* | good-luxuriant |
| 7. | *Pencillium notatum ATCC 10108* | good-luxuriant |
| 8. | *Saccharomyces cerevisiae ATCC 9763* | good-luxuriant |

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

**Test Procedure**

1. Pour plates : pipette 1 ml aliquots of a suitable series of dilution to Petri plates. Add 10-12 ml of molten medium, cooled at 45°C, and mix thoroughly.
   Spread plates: spread 0.1ml of aliquots of a suitable series of dilution over the surface of the solidified medium in a Petri dish.
2. Incubate for upto 5 days at 25°C.

**Results**

1. Colonies of yeast appear pink. Molds will grow as filamentous colonies, with various shades of pink on the reverse.

2. The number of yeasts or moulds is calculated per 1 gram or 1 ml of sample to be tested by multiplying the number of colonies by dilution factor.
3. Colonies of bacteria and yeasts could be confused by appearance and thus should be examined microscopically.

**Rose Bengal Chloramphenicol Agar (DM346)**
*Aspergillus brasiliensis ATCC 16404*

**Storage**
Store the sealed bottle containing the dehydrated medium at 15-25°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 the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

**Limitations of the Procedure**
1. Although this medium is selective primarily for fungi, microscopic examination is recommended for presumptive identification. Biochemical testing using pure cultures is required for complete identification.
2. Due to the selective properties of this medium and the type of specimen being cultured, some strains of fungi may be encountered that fail to grow or grow poorly on the complete medium; similarly, some strains of bacteria may be encountered that are not inhibited or only partially inhibited.
3. Care should be taken not to expose this medium to light, since photo degradation of rose bengal yields compounds that are toxic to fungi.

**Packaging**
**Product Name : Rose Bengal Chloramphenicol Agar**
**Product Code : DM346**

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**Available Pack sizes : 100gm/ 500gm**

**References**


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**Further Information**

For further information please contact your local MICROMASTER Representative.


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