

# PRODUCT SPECIFICATION SHEET

## Rose Bengal Agar Base (DM327)

### Intended Use

Rose Bengal Agar Base (DM326) is recommended for selective isolation and enumeration of yeast and mould from environmental material and food stuff.

### 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.<sup>(1-3)</sup> 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,<sup>(4,5)</sup> fail to completely inhibit bacterial growth<sup>(5)</sup> and have little effect in restricting the size of mold colonies.<sup>(6)</sup> Smith and Dawson<sup>(7)</sup> 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.<sup>(4, 8-11)</sup> Rose Bengal Agar Base supplemented with Rose Bengal Chloramphenicol is a modification of the Rose Bengal Chlortetracycline Agar formula of Jarvis.<sup>(11)</sup> Chloramphenicol is employed instead of chlortetracycline, in this medium as a selective supplement. Of the antibiotics most frequently employed in media of neutral pH, chloramphenicol is recommended because of its heat stability and broad antibacterial spectrum. A modified formulation of Rose Bengal Agar is recommended in *Standard Methods for the Examination of Water and Wastewater* for the enumeration of yeasts and molds.<sup>(12)</sup>

### Principles of the Procedure

Rose Bengal Agar Base contains papaic digest of soyabean meal 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 Selective Supplement (MS057) inhibit bacteria.

### Formula / Liter

Ingredients	Gms / Liter
Papaic digest of soyabean meal	5.00
Dextrose	10.00
Monopotassium phosphate	1.00
Magnesium sulphate	0.50
Rose Bengal	0.05
Agar	15.00
Final pH: 7.2 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

### Precautions

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

### Directions

1. Suspend 31.55 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. Cool to 40-45°C and add 2 ml of rehydrated Chloramphenicol Selective Supplement (MS057) for each 500 ml of medium.
5. Mix thoroughly and pour into sterile Petri plates.

# PRODUCT SPECIFICATION SHEET

## Quality Control Specifications

<b>Dehydrated Appearance</b>	Light yellow to pink homogeneous free flowing powder
<b>Prepared Medium</b>	Deep pink coloured clear to very slightly opalescent gel forms in Petri plates
<b>Reaction of 3.16% Solution</b>	pH : 7.2 ± 0.2 at 25°C
<b>Gel Strength</b>	Firm, comparable with 1.5% Agar gel

**Expected Cultural Response:** Cultural characteristics observed after an incubation at 20-25°C for 5 days with added Chloramphenicol Selective Supplement (MS057).

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Recovery
1.	<i>Aspergillus brasiliensis</i> ATCC 16404	50 -100	good	--
2.	<i>Candida albicans</i> ATCC 10231	50 -100	good	≥50%
3.	<i>Escherichia coli</i> ATCC 25922	≥10 <sup>3</sup>	inhibited	0%
4.	<i>Micrococcus luteus</i> ATCC 10240	≥10 <sup>3</sup>	inhibited	0%
5.	<i>Saccharomyces cerevisiae</i> ATCC 9763	50 -100	good	≥50%

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



**Rose Bengal Agar (DM327)**  
*Candida albicans* ATCC 10231

## 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. Calculate the number of yeasts or moulds per 1 gram or 1 ml by multiplying the number of colonies by dilution factor.
2. Colonies of yeast appear pink due to uptake of rose bengal.

## Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°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

## PRODUCT SPECIFICATION SHEET

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 Agar Base**

**Product Code : DM327**

**Available Pack sizes : 500gm**

### References

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### Further Information

For further information please contact your local MICROMASTER Representative.



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