

## Rose Bengal Agar Base

### Intended Use

Rose Bengal Agar Base is recommended for selective isolation and enumeration of Yeasts and Moulds from environmental materials and food stuffs.

### Summary

Rose Bengal Agar is a selective medium to detect and enumerate yeasts and moulds in food samples. The use of media with an acidic pH that selectively inhibits the growth of bacteria and thereby promotes the growth of fungi has been widely employed. Neutral pH media with antibiotics is advantageous for fungal growth compared to acidified media as the later may inhibit fungal growth or fail to inhibit bacterial growth and may restrict the size of mould colonies. Smith and Dawson 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 moulds from soil, sewage and foodstuffs.

Rose Bengal Agar Base supplemented with chloramphenicol is a modification of the Rose Bengal Chlortetracycline Agar formula of Jarvis. Instead of chlortetracycline, chloramphenicol is employed in this medium as a selective supplement. Chloramphenicol is recommended because of its heat stability and broad antibacterial spectrum. Rose Bengal Agar is recommended in standard methods for the enumeration of yeasts and moulds from foodstuffs and water.

### Principle

Papaic digest of soyabean meal 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 moulds. Rose Bengal is taken up by yeast and mould colonies, thereby facilitating their recognition and enumeration. Chloramphenicol Selective Supplement inhibit bacteria.

### Formula\*

Ingredients	g/L
Papaic Digest of Soyabean Meal	5.0
Dextrose	10.0
Monopotassium Phosphate	1.0
Magnesium Sulphate	0.5
Rose Bengal	0.05
Agar	15.0
Final pH (at 25°C)	7.2 ± 0.2

\*Adjusted to suit performance parameters.

### Storage and Stability

Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

### Type of Specimen

Food and Dairy samples; Soil and Water samples.

### Specimen Collection and Handling

Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

## Directions

1. Suspend 31.55 g of the powder in 1000 mL purified / distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. DO NOT OVERHEAT.
4. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
5. Cool to 40°C-45°C and add 2 mL of rehydrated Chloramphenicol Selective Supplement (204031030005) for each 500 mL of Rose Bengal Agar Base.
6. Mix thoroughly and pour into sterile petridishes.

## Quality Control

**Dehydrated Appearance:** Light pink to pink coloured, homogenous, free flowing powder.

**Prepared Appearance:** Pink to deep pink coloured, very slightly opalescent gel forms in petridishes.

**Growth Promotion Test:** Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP/IP and growth is observed after 30°C-35°C for 48 hours for bacteria and at 20°C-25°C for ≤ 5 days for fungi.

**Growth Promoting Properties:** The test results observed are within the specified temperature and shortest period of time specified in the test, inoculating ≤ 100 cfu of appropriate microorganism at 20°C-25°C for ≤ 5 days.

**Inhibitory Properties:** No growth of the test microorganism occurs for the specified temperature and not less than the longest period of the time specified, inoculating > 100 cfu of the appropriate microorganism at 30°C-35°C for ≥ 48 hours.

Organism (ATCC)	Growth
<i>Aspergillus brasiliensis</i> WLRI 034(120) (16404)	Good
<i>Candida albicans</i> 3147 (10231)	Good
<i>Saccharomyces cerevisiae</i> NRRL Y-567 (9763)	Good
<i>Escherichia coli</i> (8739)	Inhibited
<i>Escherichia coli</i> (25922)	Inhibited
<i>Micrococcus luteus</i> (9341)	Inhibited

**Note:** For good growth - Growth obtained on test media should not differ by a factor greater than 2 from calculated value for a standardized inoculum. For inhibition no growth of test microorganism should occur.

## Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

## Precautions / Limitations

1. Due to the selective properties of this medium and the Type of Specimen being cultured, some strains of fungi may grow poorly or fail to grow on the complete medium; similarly, some strains of bacteria may also not be inhibited or only partially inhibited.
2. Care should be taken not to expose this medium to light, since photodegradation of Rose Bengal yields compounds that are toxic to fungi.

## Warranty












This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

## Reference

1. Mossel D. A. A., Visser M. and Mengerink W. H. J., 1962, Lab Practice 11:109.
2. Beuchat L. R. and Cousin M. A., 2001, In Downes F. P. and Ito K., (Eds.), Compendium of Methods for the Microbiological Examination of Foods, 4<sup>th</sup> Ed., American Public Health Association, Washington, D.C.
3. Clesceri L. S., Greenberg A. E. and Eaton A. D., (Eds.), 1998, Standard Methods for the Examination of Water and Wastewater, 20<sup>th</sup> Ed., American Public Health Association, Washington, D.C.
4. Cooke W. B., 1954, Antibiot. and Chemother., 4:657.
5. Jarvis B., 1973, J. Appl. Bacteriol., 36:723.
6. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

## Product Presentation:

Cat No.	Product description	Pack Size
201180130100	Dehydrated Culture Media	100 g
201180130500	Dehydrated Culture Media	500 g

 Temperature Limit	 Manufacturer	 Batch Code	 Date of Manufacture	 This way up	 Received on
 Catalogue Number	 Consult Instructions for use	 Use-by Date	 Hygroscopic keep container tightly closed	 Opened on	

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## Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.