

## ROSE BENGAL CHLORAMPHENICOL AGAR (7664)

### Intended Use

**Rose Bengal Chloramphenicol Agar** is used for the selective isolation and enumeration of yeasts and molds from foods.

### Product Summary and Explanation

Rose Bengal Chloramphenicol Agar is a selective medium for the enumeration of fungi. In 1944, Smith and Dawson used Rose Bengal for the selective isolation of fungi from soil samples.<sup>1</sup> This formula was prepared with a neutral pH, deviating from the usual acidified medium. Several investigations have found a neutral pH with the addition of a selective agent have been successful in supporting fungal growth and restricting bacterial growth.<sup>2,3</sup> Rose Bengal Agar supplemented with Chloramphenicol is a modification of the Rose Bengal Chlorotetracycline Agar formula of Jarvis.<sup>4</sup> Chloramphenicol is recommended as the selective agent in fungal medium with a neutral pH because of its heat stability and broad antibacterial spectrum.<sup>5</sup>

Rose Bengal Chloramphenicol Agar is recommended in standard methods for the enumeration of yeast and molds from foods and water.<sup>5-7</sup> Rose Bengal Chloramphenicol Agar is also referred to as Rose Bengal Agar and Rose Bengal-Malt Extract Agar.

### Principles of the Procedure

Enzymatic Digest of Soybean Meal provides the nitrogen and vitamin sources required for organism growth in Rose Bengal Chloramphenicol Agar. The high concentration of Dextrose is included as an energy source. Monopotassium Phosphate is a buffering agent. Magnesium Sulfate provides trace elements. Rose Bengal is included as a selective agent to inhibit bacterial growth and restricts the growth of rapidly growing molds. Rose Bengal is incorporated in the cells of yeasts and molds, turning these colonies pink. Chloramphenicol is a broad-spectrum antibiotic inhibitory to a wide range of Gram-negative and Gram-positive bacteria. Agar, Bacteriological is the solidifying agent.

### Formula / Liter

Enzymatic Digest of Soybean Meal .....	5 g
Dextrose.....	10 g
Monopotassium Phosphate .....	1 g
Magnesium Sulfate .....	0.5 g
Rose Bengal .....	0.05 g
Chloramphenicol .....	0.1 g
Agar, Bacteriological .....	15.5 g

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.
2. TOXIC. Toxic by inhalation, if swallowed, or absorbed through the skin. Irritating to eyes, respiratory system, and skin. Possible risk of harm to unborn child. Possible carcinogen.

### Directions

1. Dissolve 32.2 grams of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

### Quality Control Specifications

**Dehydrated Appearance:** Powder is homogeneous, free flowing, and pink.

**Prepared Appearance:** Prepared medium is trace to slightly hazy and bright pink.

**Expected Cultural Response:** Cultural response on Rose Bengal Chloramphenicol Agar was incubated aerobically at 25 - 30°C and examined for growth at 2 – 7 days.

Microorganism	Approx. Inoculum (CFU)	Expected Results
<i>Aspergillus niger</i> ATCC® 16404	Point inoculation	Growth; reduced colony diameter
<i>Escherichia coli</i> ATCC® 25922	300 - 1000	Inhibited
<i>Enterococcus faecalis</i> ATCC® 29212	300 - 1000	Inhibited
<i>Mucor racemosus</i> ATCC® 42647	Point inoculation	Growth
<i>Penicillium roquefortii</i> ATCC® 10110	Point inoculation	Growth; reduced colony diameter
<i>Saccharomyces cerevisiae</i> ATCC® 9763	10 - 300	Growth; pink colonies; may have reduced recovery

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

### Test Procedure

Consult appropriate references for recommended test procedures on the isolation and identification of yeasts and molds.

### Results

Colonies of yeast appear pink. Molds will grow as filamentous colonies, with various shades of pink. Count plates containing 15 to 150 colonies and report as colony forming units (CFU) per gram or mL of sample. Refer to appropriate references for a complete discussion on yeast and molds.

### Storage

Store sealed bottle containing the dehydrated medium at 2 - 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 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. Some strains may be encountered that grow poorly or fail to grow on this medium.
2. Antimicrobial agents added to medium inhibit bacteria may also inhibit certain pathogenic fungi.
3. Exposure to light on Rose Bengal Chloramphenicol Agar should be avoided. Photodegradation of Rose Bengal can produce compounds that are toxic to fungi.

### Packaging

<b>Rose Bengal Chloramphenicol Agar</b>	<b>Code No.</b>	<b>7664A</b>	<b>500 g</b>
		<b>7664B</b>	<b>2 kg</b>
		<b>7664C</b>	<b>10 kg</b>

### References

1. **Smith, N. R. and V. T. Dawson.** 1944. The bacteriostatic action of rose bengal in media used for the plate counts of soil fungi. *Soil Sci.* **58**:467-471.
2. **Mossel, D. A. A., M. Visser, and W. H. J. Mengerink.** 1962. *Lab. Pract.* **11**:109-112.
3. **Koburger, J. A.** 1968. *Bact. Proc.* **13**:A73.
4. **Jarvis, B.** 1973. Comparison of an improved rose bengal-chlortetracycline agar with other media for the selective isolation and enumeration of molds and yeasts in foods. *J. Appl. Bact.* **36**:723-727.
5. **Mislivec, P. B., L. R. Beuchat, and M. A. Cousin.** 1992. Yeast and Molds. *In* C. Vanderzant and D. F. Splittstoesser (eds.), *Compendium of methods for the microbiological examination of foods*, 3rd ed. American Public Health Assoc., Washington, D.C.
6. **Marshall, R. T. (ed.).** 1993. *Standard methods for the examination of dairy products*, 16<sup>th</sup> ed. American Public Health Assoc., Washington, D.C.
7. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. *Standard methods for the examination of water and wastewater*, 19<sup>th</sup> ed. American Public Health Assoc., Washington, D.C.

### Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.