

## DRBC AGAR (7591)

### Intended Use

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

### Product Summary and Explanation

DRBC Agar is based on Dichloran Rose Bengal Chlortetracycline (DRBC) Agar formula described by King, Hocking, and Pitt.<sup>1</sup> DRBC Agar conforms with APHA guidelines for the mycological examination of foods containing chloramphenicol rather than chlortetracycline as proposed by King, Hocking, and Pitt.<sup>2</sup> DRBC Agar is a selective medium, supporting good growth of yeasts and molds.

### Principles of the Procedure

Peptone provides nitrogen, carbon, and vitamins required for organism growth. Glucose is included as an energy source. Monopotassium Phosphate is a buffering agent. Magnesium Sulfate is a source of divalent cations and sulfate. The antifungal agent, Dichloran, is added to reduce colony diameters of spreading fungi. The pH of the medium is reduced from 7.2 to 5.6 for improved inhibition of spreading fungi.<sup>1</sup> Rose Bengal suppresses growth of bacteria and restricts the size and height of colonies of more rapidly growing molds. The concentration of Rose Bengal is reduced from 50 µg/mL to 25 µg/mL, found in Rose Bengal Chloramphenicol Agar, for optimal performance with Dichloran. Chloramphenicol is included to inhibit the growth of bacteria present in environmental and food samples. Inhibition of bacterial growth and the restricted spreading of rapidly growing molds aids in isolation of slow-growing fungi. In addition, Rose Bengal is absorbed by yeast and mold colonies, allowing these colonies to be easily recognized and enumerated. Reduced recovery of yeasts may be encountered due to increased activity of Rose Bengal at pH 5.6.<sup>1</sup> Agar is the solidifying agent.

### Formula / Liter

Enzymatic Digest of Animal Tissue .....	5 g
Glucose .....	10 g
Monopotassium Phosphate .....	1 g
Magnesium Sulfate .....	0.5 g
Rose Bengal .....	0.025 g
Dichloran .....	0.002 g
Chloramphenicol .....	0.1 g
Agar .....	15 g

Final pH: 5.6 ± 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 and if swallowed. May cause cancer. Irritating to eyes, skin, and respiratory system.

### Directions

1. Suspend 31.6 g 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. DO NOT OVERHEAT.

### 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 DRBC Agar incubated aerobically at 25 - 30°C and examined for growth after 2 - 7 days.

Microorganism	Approx. Inoculum (CFU)	Expected Results
<i>Aspergillus niger</i> ATCC® 16404	Point inoculation	Growth, reduced colony diameter
<i>Bacillus subtilis</i> ATCC® 9372	~ 1000	Inhibited
<i>Candida albicans</i> ATCC® 10231	10 - 300	Growth, may have reduced recovery
<i>Escherichia coli</i> ATCC® 25922	~ 1000	Inhibited
<i>Mucor racemosus</i> ATCC® 42647	Point Inoculation	Growth, reduced colony diameter
<i>Penicillium roquefortii</i> ATCC® 10110	Point Inoculation	Growth, reduced colony diameter
<i>Saccharomyces cerevisiae</i> ATCC® 9763	10 - 300	Growth, may have reduced recovery

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

### Test Procedure

1. Inoculate 0.1 mL of appropriate decimal dilutions of the sample in duplicate onto the surface of DRBC Agar plates. The plates should be dried overnight at room temperature. Spread the inoculum over the entire surface of plate using a sterile, bent-glass rod.
2. Incubate plates upright at 22 - 25°C. Examine for growth of yeasts and molds after 3, 4, and 5 days incubation.

### Results

Colonies of mold and yeast should be apparent within 5 days incubation. Colonies of yeast appear pink from the absorption of Rose Bengal. Report results as colony forming units per gram or milliliter of sample.

### 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 by keeping container tightly closed.

### Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

### Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

### Packaging

DRBC Agar

Code No.	57591A	500 g
	7591B	2 kg
	7591C	10 kg

### References

1. King, A. D., A. D. Hocking, and J. I. Pitt. 1979. Dichloran-rose bengal medium for the enumeration and isolation of molds from foods. *Appl. Environ. Microbiol.* **37**:959-964.
2. Mislivec, P. B., L. R. Beuchat, and M. A. Cousin. 1992. Yeasts and molds, p. 239-249. In C. Vanderzant, and D. F. Splittstoesser, (eds.). *Compendium of methods for the microbiological examination of foods*, 3<sup>rd</sup> ed. American Public Health Association, Washington, D.C.
3. Vanderzant, C. and D. F. Splittstoesser, (eds.). 1992. *Compendium of methods for the microbiological examination of foods*, 3<sup>rd</sup> ed. American Public Health Association, 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.