

## DC MEDIUM with BCIG (7704)

### Intended Use

**DC Medium with BCIG** is used in the chromogenic differentiation of *E. coli* from other coliforms in water samples using the membrane filtration method.

### Product Summary and Explanation

DC (Differential Coliform) Medium with BCIG is a selective and differential medium for the presumptive identification of *E. coli*. This medium is enhanced by the addition of a chromogenic agent, BCIG, 5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronide to detect glucuronidase activity. The presence of the enzyme  $\beta$ -D-glucuronidase differentiates most *E. coli* spp. from other coliforms, and is the same enzyme used in the MUG reaction.<sup>1</sup> BCIG reacts slightly differently, and when released into the medium is insoluble, accumulating within the cell, imparting a blue to purple color to presumptive *E. coli* colonies.

### Principles of the Procedure

Tryptose and Proteose Peptone provide nitrogen, carbon, and amino acids in DC Medium with BCIG. Yeast Extract supplies vitamins and minerals. Sodium Chloride maintains the osmotic balance of the medium. Bile Salts is a selective agent against Gram-positive bacteria, particularly bacilli and fecal streptococci. Cefsulodin supplements the medium as a selective agent. *E. coli* absorbs the chromogenic substrate, 5-bromo-4-chloro-3-indolyl- $\beta$ -D-glucuronide, BCIG. The enzyme  $\beta$ -glucuronidase splits the bond between the chromophore 5-bromo-4-chloro-3-indolyle and the  $\beta$ -D-glucuronide. The released chromophore is colored and accumulates within the cells imparting a blue to purple colony color. Neutral Red is the dye indicator, and Agar is the solidifying agent.

### Formula / Liter

Lactose .....	10.0 g
Tryptose .....	10.0 g
Yeast Extract.....	3.0 g
Sodium Chloride .....	5.0 g
Proteose Peptone .....	5.0 g
Bile Salts .....	1.5 g
BCIG .....	0.2 g
Neutral Red.....	0.08 g
Agar .....	15.0 g

Final pH: 7.2  $\pm$  0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

### Supplement

Cefsulodin solution, 10 mg/mL

### Precautions

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

### Directions

1. Dissolve 49.8 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. DO NOT AUTOCLAVE.
4. Cool to 45 - 50°C.
5. Aseptically add 1.2 mL of a sterile 10 mg/mL solution of Cefsulodin and mix well.

### Quality Control Specifications

**Dehydrated Appearance:** Powder is homogeneous, free flowing, and beige to grayish pink with blue specs.

**Prepared Appearance:** Prepared medium is clear to trace hazy and red.

**Expected Cultural Response:** Cultural response on DC Medium with BCIG using the Membrane Filtration Method. Cultures were incubated at 35 ± 2°C and examined for growth after 22 - 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results	Expected Reactions
<i>Escherichia coli</i> ATCC® 25922	10 - 100	Growth	Blue / purple colonies
<i>Citrobacter freundii</i> ATCC® 8090	10 - 100	Growth	Pink colonies or white w/ pink center colonies
<i>Proteus mirabilis</i> ATCC® 12453	10 - 100	Growth	Small pale salmon colonies
<i>Staphylococcus aureus</i> ATCC® 25923	1000	Inhibited	---

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

### **Test Procedure**

#### **Membrane Filtration Technique**

1. Filter appropriate aliquot of the sample(s) to be tested through a cellulose membrane.
2. Transfer the membrane to a prepared plate of DC Medium with BCIG, supplemented with Cefsulodin.
3. Incubate at 35°C for 22 – 24 hours.
4. Examine plates for growth of *E. coli* colonies.

For a complete discussion on Membrane Filtration Procedures, refer to appropriate references.<sup>2,3</sup>

### **Results**

*E. coli* colonies are blue/purple. The total number of *E. coli* per gram can be calculated by multiplying the blue/purple colonies by the dilution factor. The number of presumptive *E. coli* is obtained by multiplying the number of blue/purple colonies by the dilution factor.

### **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 the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

### **Limitation of the Procedure**

1. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow.

### **Packaging**

**DC Medium with BCIG**

<b>Code No.</b>	<b>7704A</b>	<b>500 g</b>
	<b>7704B</b>	<b>2 kg</b>
	<b>7704C</b>	<b>10 kg</b>

### **References**

1. Feng, P. C. S., and P. A. Hartmann. 1982. Appl. Environ. Microbiol. **43**:1320-1329.
2. [www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm](http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm).
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.