



## **BILE ESCULIN AGAR (7249)**

### **Intended Use**

**Bile Esculin Agar** is used for the selective isolation and differentiation of group D streptococci in a laboratory setting. Bile Esculin Agar is not intended for use in the diagnosis of disease or other conditions in humans.

### **Product Summary and Explanation**

Bile Esculin Agar is based on the formulation described by Swan and further evaluated by Facklam and Moody.<sup>1,2</sup> Rochaix first noted the value of esculin hydrolysis in the identification of enterococci.<sup>3</sup> Meyer and Schonfeld added bile to the esculin medium and demonstrated 61 of 62 enterococci strains were able to grow and hydrolyze esculin, while other streptococci could not.<sup>4</sup>

Molecular taxonomic studies of the genus *Streptococcus* have placed enterococci, previously described as group D streptococci, in the genus *Enterococcus*.<sup>5</sup> The ability to hydrolyze esculin in the presence of bile is a characteristic of enterococci and group D streptococci. Swan compared the use of an esculin medium containing 40% bile salts with the Lancefield serological method of grouping,<sup>1</sup> and reported that a positive reaction on the bile esculin medium correlated with a serological group D precipitin reaction. Facklam and Moody found that the bile esculin test provided a reliable means of identifying group D streptococci and differentiating them from non-group D streptococci.<sup>2</sup>

Bile Esculin Agar is in standard procedures for the microbiological examination of food products.<sup>6-8</sup>

### **Principles of the Procedure**

Organisms positive for esculin hydrolysis hydrolyze the esculin to esculetin and dextrose. The esculetin reacts with the ferric citrate to form a dark brown or black complex. Oxbile is used to inhibit Gram-positive bacteria other than enterococci. Beef Extract and Enzymatic Digest of Gelatin are the carbon and nitrogen sources used for general growth requirements in Bile Esculin Agar. Agar is the solidifying agent.

### **Formula / Liter**

Beef Extract .....	11 g
Enzymatic Digest of Gelatin.....	34.5 g
Esculin .....	1 g
Oxbile.....	2 g
Ferric Ammonium Citrate.....	0.5 g
Agar .....	15 g

Final pH: 6.6 ± 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 64 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.

### **Quality Control Specifications**

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

**Prepared Appearance:** Prepared medium is trace to slightly hazy, opalescent, and grey-yellow.

**Expected Cultural Response:** Cultural response on Bile Esculin Agar at 35 ± 2°C and examined for growth after 18 - 24 hours incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reaction
<i>Enterococcus faecalis</i> ATCC® 19433	10 - 300	Fair to good	Black colonies
<i>Enterococcus faecalis</i> ATCC® 29212	10 - 300	Fair to good	Black colonies
<i>Enterococcus faecalis</i> ATCC® 33186	10 - 300	Fair to good	Black colonies
<i>Escherichia coli</i> ATCC® 25922	10 - 300	Fair to good	--
<i>Streptococcus pyogenes</i> ATCC® 19615	10 - 300	None to poor	--

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

### **Test Procedure**

Refer to appropriate references for instructions on specific material being tested for group D streptococci.

### **Results**

Refer to appropriate references and procedures for results.

### **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 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 varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

### **Packaging**

<b>Bile Esculin Agar</b>	<b>Code No.</b>	<b>7249A</b>	<b>500 g</b>
		<b>7249B</b>	<b>2 kg</b>
		<b>7249C</b>	<b>10 kg</b>

### **References**

1. **Swan, A.** 1954. The use of bile-esculin medium and of Maxted's technique of Lancefield grouping in the identification of enterococci (group D streptococci). *J. Clin. Pathol.* **7**:160.
2. **Facklam, R. R., and M. D. Moody.** 1970. Presumptive identification of group D streptococci: the bile-esculin test. *Appl. Microbiol.* **20**:245.
3. **Rochaix, A.** 1924. Milieux a leculine pour le diagnostid differentiel des bacteries du grojps strepto-entero-pneumocoque. *Comt. Rend. Soc. Biol.* **90**:771-772.
4. **Meyer, K., and H. Schönfeld.** 1926. Über die Unterscheidung des *Enterococcus* vom *Streptococcus viridans* und die Beziehung beider zum *Strptococcus lactis*. *Zentralb. Bakteriol Parasitenkd. Infektionskr. Hyg. Abt. I orig.* **99**:402-416.
5. **Schleifer, K. H., and R. Kilpper-Balz.** 1987. Molecular and chemotaxonomic approaches to the classification of streptococci, enterococci and lactococci: a review. *Syst. Appl. Microbiol.* **10**:1-19.
6. **Vanderzant, C., 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.
7. **Bacteriological Analytical Manual.** 1995. 8<sup>th</sup> ed. AOAC International, Gaithersburg, MD.
8. **Marshall, R. T. (ed.).** 1992. *Standard methods for the examination of dairy products*, 16<sup>th</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.