

## PRESENCE-ABSENCE BROTH (7500)

### Intended Use

**Presence-Absence Broth** is used for the detection of coliform bacteria in water treatment plants or distribution systems using the presence-absence coliform test.

### Product Summary and Explanation

The Presence-Absence (P-A) test is a presumptive detection for coliforms in water. The test is a simple modification of the multiple-tube procedure.<sup>1</sup> One 100 mL test sample is inoculated into a single culture bottle to obtain qualitative information on the presence or absence of coliforms, through the presence or absence of lactose fermentation.<sup>1</sup> This test is based on the principle that coliforms and other pollution indicator organisms should not be present in a 100 mL water sample.<sup>2-4</sup>

Comparative studies with the membrane filter procedure indicate the P-A test may maximize coliform detection in samples containing many organisms that could overgrow coliform colonies and cause problems in detection.<sup>1</sup> The P-A test is described in standard methods for water testing<sup>1</sup> and U.S. EPA.<sup>5</sup>

### Principles of the Procedure

The nitrogen, vitamin, and amino acids sources are provided by Enzymatic Digest of Gelatin, Enzymatic Digest of Casein, and Beef Extract. Lactose is the fermentable carbohydrate. Dipotassium Phosphate and Monopotassium Phosphate provide buffering capacity. Sodium Chloride maintains the osmotic balance of the medium. Sodium Lauryl Sulfate is the selective agent, inhibiting many organisms except coliforms. Bromcresol Purple is used as an indicator dye; lactose-fermenting organisms turn the medium from purple to yellow with or without gas production.

### Formula / Liter

Beef Extract .....	3 g
Enzymatic Digest of Gelatin .....	5 g
Lactose .....	7.46 g
Enzymatic Digest of Casein .....	9.83 g
Dipotassium Phosphate .....	1.35 g
Monopotassium Phosphate .....	1.35 g
Sodium Chloride .....	2.46 g
Sodium Lauryl Sulfate .....	0.05 g
Bromcresol Purple .....	0.0085 g

Final pH: 6.8 ± 0.2 at 25°C

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

### Precautions

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

### Directions

1. Prepare triple strength concentration by adding 91.5 g of the medium in one liter of purified water.
2. Mix thoroughly.
3. Dispense 50 mL into a 250 mL screw-cap milk dilution bottle.
4. Autoclave at 121°C for 12 minutes.
5. Cool and add 100 mL water sample.

### Quality Control Specifications

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

**Prepared Appearance:** Prepared medium is clear, very dark to red-purple, and none to light precipitate.

**Expected Cultural Response:** Cultural response in Presence-Absence Broth incubated aerobically at  $35 \pm 2$  °C and examined for growth after 18 - 48 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reaction
<i>Enterococcus faecalis</i> ATCC® 29212	10 - 300	Good to excellent	No change (purple to light grey-yellow)
<i>Escherichia coli</i> ATCC® 25922	10 - 300	Good to excellent	Yellow
<i>Escherichia coli</i> ATCC® 11775	10 - 300	Good to excellent	Yellow
<i>Pseudomonas aeruginosa</i> ATCC® 27853	10 - 300	Partial inhibition	No change (Purple to grey-purple)

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

### **Test Procedure**

1. Inoculate 50 mL of the sterile triple strength P-A Broth with 100 mL of the water sample.
2. Invert the bottle a few times to achieve an even distribution of the medium throughout the test sample. Incubate at  $35 \pm 0.5$ °C.
3. Inspect for acid and gas production after 24 and 48 hours incubation.

### **Results**

An acid reaction from lactose fermentation is indicated by a distinct yellow color in the medium. Gas production is indicated by bubbles or foam present in the medium. Any amount of gas and/or acid is a positive presumptive test requiring confirmation.<sup>1</sup> Report results as positive or negative for coliforms per 100 mL 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.

### **Expiration**

Refer to expiration date stamped on 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.

### **Limitations of the Procedure**

1. The P-A test is only a presumptive test for coliforms.
2. Confirmation and differentiation of coliforms detected by the P-A test may be achieved through biochemical testing, incubation time, and temperatures as outlined in appropriate references.<sup>1,5</sup>
3. Extending P-A test incubation period to 72 or 96 hours will allow isolation of other indicator organisms. However, indicator bacteria isolated after 48 hours incubation may not be considered for regulatory purposes.

### **Packaging**

Presence-Absence Broth	Code No.	7500A	500 g
		7500B	2 kg
		7500C	10 kg

### **References**

1. 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 Association, Washington, D.C.
2. Clark, J. A., and J. E. Pagel. 1977. Pollution indicator bacteria associated with municipal raw and drinking water supplies. Can. J. Microbiol. **23**:465-470.
3. Clark, J. A. 1980. The influence of increasing numbers of nonindicator organisms upon the detection of indicator organisms by the membrane filter and presence-absence tests. Can. J. Microbiol. **26**:827-832.
4. Clark, J. A., C. A. Burger, and L. E. Sabatinos. 1982. Characterization of indicator bacteria in municipal raw water, drinking water and new main water samples. Can. J. Microbiol. **28**:1002-1013.
5. Federal Register. 1989. National primary drinking water regulations; total coliforms (including fecal coliforms and *E. coli*). Fed Regist. **54**:27544-27568.

### **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.