



## EC MEDIUM (7206)

### Intended Use

**EC Medium** is used for the detection of coliform bacteria at 37°C and *Escherichia coli* at elevated temperatures (44.5 and 45.5°C).

### Product Summary and Explanation

EC Medium was developed by Hajna and Perry<sup>1</sup> in an effort to improve the methods for the detection of the coliform group and *E. coli*. This medium consists of a buffered lactose broth with the addition of 0.15% Bile Salts Mixture. Growth of spore-forming bacteria and fecal streptococci are inhibited by the bile salts, while growth of *E. coli* is enhanced by its presence. This medium can be used at 37°C for the detection of coliform organisms or at 44.5 or 45.5°C for the isolation of *E. coli*.

EC Medium is employed in elevated-temperature tests for distinguishing organisms of the total coliform group that also belong to the fecal coliform group.<sup>2</sup> The fecal coliform test, using EC Medium, is applicable to investigations of drinking water, stream pollution, raw water sources, wastewater treatment systems, bathing waters, seawaters, and general water-quality monitoring. EC Medium is used in the MPN (Most Probable Number) test for fecal coliforms. EC Medium should not be used for the direct isolation of coliforms; prior enrichment in presumptive media is required for optimum recovery of fecal coliforms. EC Medium is used in methods for food and water testing.<sup>2-5</sup>

### Principles of the Procedure

Tryptose, a mixed enzymatic digest of protein, provides nitrogen, vitamins, and amino acids in EC Medium. Lactose is the carbon source. Bile Salts Mixture is the selective agent against Gram-positive bacteria, particularly bacilli and fecal streptococci. Dipotassium Phosphate and Monopotassium Phosphate are the buffering agents. Sodium Chloride maintains the osmotic balance of the medium.

### Formula / Liter

|                               |       |
|-------------------------------|-------|
| Tryptose .....                | 20 g  |
| Lactose .....                 | 5 g   |
| Bile Salts Mixture .....      | 1.5 g |
| Dipotassium Phosphate .....   | 4 g   |
| Monopotassium Phosphate ..... | 1.5 g |
| Sodium Chloride .....         | 5 g   |

Final pH: 6.9 ± 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. Dissolve 37 g of the medium in one liter of purified water.
2. Mix thoroughly.
3. Distribute into tubes containing inverted fermentation Durham tubes.
4. 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 brilliant to clear.

**1X Solution:** Gold

**2X Solution:** Medium to dark orange amber

**Precipitate:** None to light

**Expected Cultural Response:** With reference to the FDA/BAM Chapter 4, Enumeration of Escherichia coli and the Coliform Bacteria (update 2/2013) and APHA Standard Methods for the Examination of Water and Wastewater 20<sup>th</sup> Edition Section 9221: Two inoculation methods can be followed:

- Direct inoculation of test strains into EC Medium with incubation at  $35 \pm 2^\circ\text{C}$  and at  $44.5 \pm 0.2^\circ\text{C}$ . Incubate cultures in an aerobic atmosphere at indicated temperature and then examine for growth and gas at  $24 \pm 2$  hours; if no gas re-incubate to  $48 \pm 3$  hours to confirm gas.
- Pre-enrich test strains in LSB (Lauryl Sulfate Broth) at  $35 \pm 2^\circ\text{C}$ . If no growth/gas after 24 hours, re-incubate up to 48 hours and transfer a loopful of LSB enriched culture to EC Medium and incubate in a water bath at 44.5 and 45.5°C aerobically. Examine for growth and gas at  $24 \pm 2$  hours; if no gas re-incubate to  $48 \pm 3$  hours to confirm gas.

| <u>MICRO-ORGANISM</u>         | ATCC                   | APPROX. INOCULUM (CFU)  | <u>EXPECTED RESULTS</u>                         |                            |   |             | <u>ACTUAL RESULTS</u>                   |  |   |  |
|-------------------------------|------------------------|---|---|----------------------------|---|-------------|---|--|---|--|
|                               |                        |   | Direct inoculation into EC at 35C/44.5C         |                            | LSB pre-enrichment at 35C; EC at 44.5/45.5C |             | Direct inoculation into EC at 35C/44.5C |  | LSB pre-enrichment at 35C; EC at 44.5/45.5C |  |
|                               |                        |   | Growth  | Gas                        | Growth                                      | Gas         |   |  |   |  |
| <i>Enterobacter aerogenes</i> | 13048                  | 10-300 for 35C; 10-300 for 44.5C; 10-100 for LSB pre-enrichment | Good to Excellent at 35C; none to poor at 44.5C | $\pm$ at 35C<br>- at 44.5C | None to Good                                | -           |   |  |   |  |
| <i>Enterococcus faecalis</i>  | 29212                  | $10^3$  | Inhibited                                       | N/A                        | Inhibited                                   | N/A         |   |  |   |  |
| <i>Escherichia coli</i>       | 25922 or 8739 or 11775 | 10-300 for 35C; $10^3$ for 44.5C; 10-100 for LSB pre-enrichment | Fair to Excellent                               | +                          | Fair to Excellent                           | Weak + to + |   |  |   |  |

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

### Test Procedure

Refer to appropriate references for specific procedures using EC Medium.<sup>2-5</sup>

### Results

Gas production with growth in EC Medium within  $48 \pm 3$  hours or less is considered a positive fecal coliform reaction. Failure to produce gas with little or no growth is a negative reaction.<sup>2</sup>

### 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

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

### Packaging

|                  |                 |              |              |
|------------------|-----------------|--------------|--------------|
| <b>EC Medium</b> | <b>Code No.</b> | <b>7206A</b> | <b>500 g</b> |
|                  |                 | <b>7206B</b> | <b>2 kg</b>  |
|                  |                 | <b>7206C</b> | <b>10 kg</b> |

### References

1. **Hajna and Perry.** 1943. Am J. Public Health. **33**:550.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1998. Standard methods for the examination of water and wastewater, 20<sup>th</sup> ed., American Public Health Association, Washington, D.C.
3. **Cunniff, P. (ed.).** 1995. Official Methods of Analysis AOAC International, 16<sup>th</sup> ed., AOAC International, Gaithersburg, MD.
4. **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.
5. **FDA/BAM Chapter 4 Enumeration of E. coli and the Coliform Bacteria** updated 2/2013; [www.fda.gov](http://www.fda.gov)

### 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.