# Letheen Agar, Modified • Letheen Broth, Modified

## Intended Use

Letheen Agar, Modified and Letheen Broth, Modified are used for the microbiological testing of cosmetics.

## Summary and Explanation

Letheen Agar, Modified and Letheen Broth, Modified are based on Letheen Agar, Modified and Letheen Broth, Modified as described in the U.S. Food and Drug Administration (FDA) *Bacteriological Analytical Manual*.[1] Letheen Agar, Modified and Letheen Broth, Modified are recommended by the FDA for use in the microbiological testing of cosmetics.[1]

## User Quality Control

### *Identity Specifications*

**Difco™ Letheen Agar, Modified**

| | |
|---|---|
| Dehydrated Appearance: | Tan, moist with a tendency to clump. |
| Solution: | 5.91% solution, soluble in purified water upon boiling. Solution is medium amber, opalescent, may have a slight precipitate. After cooling, slightly opalescent. |
| Prepared Appearance: | Light-medium amber, slightly opalescent, may have a slight, fine precipitate. |
| Reaction of 5.91% Solution at 25°C: | pH 7.2 ± 0.2 |

**Difco™ Letheen Broth, Modified**

| | |
|---|---|
| Dehydrated Appearance: | Tan, homogeneous, appears moist with a tendency to clump. |
| Solution: | 4.28% solution, soluble in purified water upon boiling. Solution is medium amber, clear to slightly opalescent, may have slight fine precipitate. |
| Prepared Appearance: | Medium-dark amber, slightly opalescent, may have a slight fine precipitate. |
| Reaction of 4.28% Solution at 25°C: | pH 7.2 ± 0.2 |

### *Cultural Response*

**Difco™ Letheen Agar, Modified or Letheen Broth, Modified**

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24-48 hours.

| ORGANISM | ATCC™ | INOCULUM CFU | RECOVERY AGAR | RECOVERY BROTH |
|---|---|---|---|---|
| *Staphylococcus aureus* | 6538 | 25-100 | Good | Good |
| *Salmonella enterica* subsp. *enterica* serotype Typhi | 6539 | 25-100 | N/A | Good |

## Principles of the Procedure

Beef extract, included in the Letheen Agar and Letheen Broth bases, and peptone provide carbon and nitrogen sources required for good growth of a wide variety of bacteria and fungi. The peptone level was increased in the modified Letheen Agar and Broth formulas to provide for better growth. Vitamins and cofactors, required for growth as well as additional sources of nitrogen and carbon, are provided by yeast extract. Sodium chloride provides a suitable osmotic environment. In Letheen Broth, Modified sodium chloride is provided by the Letheen Broth component. Both media also contain polysorbate 80, lecithin and sodium bisulfite to partially neutralize the preservative systems commonly found in cosmetics. Additional agar is included in Letheen Agar, Modified as the solidifying agent.

## Formulae

**Difco™ Letheen Agar, Modified**

| Approximate Formula* Per Liter | | |
|---|---|---|
| Letheen Agar | 32.0 | g |
| Tryptone | 5.0 | g |
| Proteose Peptone No. 3 | 10.0 | g |
| Yeast Extract | 2.0 | g |
| Sodium Chloride | 5.0 | g |
| Sodium Bisulfite | 0.1 | g |
| Agar | 5.0 | g |

**Difco™ Letheen Broth, Modified**

| Approximate Formula* Per Liter | | |
|---|---|---|
| Letheen Broth | 25.7 | g |
| Tryptone | 5.0 | g |
| Proteose Peptone No. 3 | 10.0 | g |
| Yeast Extract | 2.0 | g |
| Sodium Bisulfite | 0.1 | g |

**Adjusted and/or supplemented as required to meet performance criteria.*

## Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   **Difco™ Letheen Agar, Modified** - 59.1 g;
   **Difco™ Letheen Broth, Modified** - 42.8 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.

3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

## Procedure[1]

1. Prepare and dilute samples in Letheen Broth, Modified in accordance with established guidelines.
2. Using the spread plate technique, inoculate in duplicate 0.1 mL of the diluted samples onto Letheen Agar, Modified, Potato Dextrose Agar (or Malt Extract Agar) containing chlortetracycline, Baird-Parker Agar (or Vogel-Johnson Agar, optional), Anaerobic Agar, and a second set of Letheen Agar, Modified plates.
3. Incubate one set of Letheen Agar, Modified plates at 30 ± 2°C for 48 hours and the other set at 35 ± 2°C under anaerobic conditions for 2-4 days. Incubate the Potato Dextrose Agar (or Malt Extract Agar) plates at 30 ± 2°C for 7 days and the Baird-Parker Agar (or Vogel-Johnson Agar) plates, if inoculated, at 35 ± 2°C for 48 hours.
4. Incubate the diluted samples from step 1 at 35 ± 2°C for 7 days. Subculture enriched samples onto Letheen Agar, Modified only if there is no growth on the primary Letheen Agar, Modified plates.

## Expected Results

Examine plates for evidence of growth and characteristic colonial morphology. Determine colony counts and subculture each colony type onto Letheen Agar, Modified and MacConkey Agar (also Baird- Parker or Vogel-Johnson Agar, if used in step 2).

Determine Gram reaction, cell morphology and catalase reactions. Identify bacterial isolates in accordance with established procedures.[1]

## Reference

1. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.

## Availability

### Difco™ Letheen Agar, Modified

BAM

Cat. No. 263110 Dehydrated – 500 g*

*Europe*

Cat. No. 257452 Sterile Pack **RODAC**™ Plates – Ctn. of 100*
257451 Prepared Plates (sterile) – Ctn. of 100*

### Difco™ Letheen Broth, Modified

BAM

Cat. No. 263010 Dehydrated – 500 g*

*Europe*

Cat. No. 257327 Prepared Bottles, 500 mL – Pkg. of 4

**Store at 2-8°C.*