

# Lethen Agar, Modified • Lethen Broth, Modified

## Intended Use

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

## Summary and Explanation

Lethen Agar, Modified and Lethen Broth, Modified are based on Lethen Agar, Modified and Lethen Broth, Modified as described in the U.S. Food and Drug Administration (FDA) *Bacteriological Analytical Manual*.<sup>1</sup> Lethen Agar, Modified and

Lethen Broth, Modified are recommended by the FDA for use in the microbiological testing of cosmetics.<sup>1</sup>

## Principles of the Procedure

Beef extract, included in the Lethen Agar and Lethen 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 Lethen 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 Lethen Broth, Modified sodium chloride is provided by the Lethen 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 Lethen Agar, Modified as the solidifying agent.

## User Quality Control

### Identity Specifications

#### Difco™ Lethen 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™ Lethen 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™ Lethen Agar, Modified or Lethen 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
<i>Staphylococcus aureus</i>	6538	25-100	Good	Good
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhi	6539	25-100	N/A	Good

## Formulae

### Difco™ Lethen Agar, Modified

Approximate Formula* Per Liter		
Lethen 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™ Lethen Broth, Modified

Approximate Formula* Per Liter		
Lethen 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

- Suspend the powder in 1 L of purified water:  
 Difco™ Lethen Agar, Modified - 59.1 g;  
 Difco™ Lethen Broth, Modified - 42.8 g.  
 Mix thoroughly.
- 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<sup>1</sup>

1. Prepare and dilute samples in Lethen Broth, Modified in accordance with established guidelines.
2. Using the spread plate technique, inoculate in duplicate 0.1 mL of the diluted samples onto Lethen Agar, Modified, Potato Dextrose Agar (or Malt Extract Agar) containing chlor-tetracycline, Baird-Parker Agar (or Vogel-Johnson Agar, optional), Anaerobic Agar, and a second set of Lethen Agar, Modified plates.
3. Incubate one set of Lethen 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 Lethen Agar, Modified only if there is no growth on the primary Lethen Agar, Modified plates.

### Expected Results

Examine plates for evidence of growth and characteristic colonial morphology. Determine colony counts and subculture each colony type onto Lethen 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.<sup>1</sup>

### Reference

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

### Availability

#### Difco™ Lethen 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™ Lethen 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.