M17 Agar • M17 Broth

Intended Use

M17 Agar is used for isolating and enumerating lactic streptococci in yogurt, cheese starters and other dairy products.

M17 Broth is used for isolating lactic streptococci from yogurt, cheese starters and other dairy products.

Summary and Explanation

Lactic streptococci are acid-producing bacteria. They are nutritionally fastidious and require complex culture media for optimum growth. One study showed that in a synthetic medium, all strains had an obligate requirement for at least six amino acids and three vitamins.[1] These homofermentative lactic streptococci produce large amounts of acid and, in a culture medium without an adequate buffering system, the pH decreases and adversely affects growth. Lowrie and Pearce[2] developed M16 Medium but it lacked a strong buffering system. Terzaghi and Sandine[3] worked with M16 Medium and demonstrated that the rapid drop in pH that accompanies lactic streptococcal growth can adversely affect colony size and phage plaque formation. They modified M16 Medium using disodium-β-glycerophosphate as a buffer and called it M17.

Shankar and Davies[4] found that disodium-β-glycerophosphate in M17 Broth suppressed *Lactobacillus bulgaricus* and selectively isolated *Streptococcus thermophilus* from yogurt. Similar results were achieved using M17 Broth solidified with agar. The International Dairy Federation recommends M17 Agar for isolating *S. thermophilus* from yogurt.[5] M17 Agar is a standard methods medium for isolating lactic streptococci.[6]

User Quality Control

Identity Specifications

Difco™ M17 Agar

Dehydrated Appearance:	Beige to medium tan, free-flowing, homogeneous.
Solution:	48.25 g soluble in 950 mL purified water upon boiling. Solution is light-medium to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, very slightly to slightly opalescent, no significant precipitate.
Reaction of 48.25 g/950 mL at 25°C:	pH 6.9 ± 0.2

Difco™ M17 Broth

Dehydrated Appearance:	Beige to medium tan, free-flowing, homogeneous.
Solution:	37.25 g soluble in 950 mL purified water upon boiling. Solution is light-medium to medium amber, clear to very slightly opalescent.
Prepared Appearance:	Light medium to medium amber, clear to very slightly opalescent, no significant precipitate.
Reaction of 37.25 g/950 mL at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ M17 Agar or M17 Broth

Prepare the medium per label directions with the addition of lactose. Inoculate and incubate for 40-48 hours; *Lactococcus lactis* subsp. *cremoris* at 30 ± 2°C and the remaining organisms at 35 ± 2°C.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Lactobacillus delbrueckii subsp. *bulgaricus*	11842	10^2-10^3	None to poor
Lactococcus lactis subsp. *cremoris*	19257	10^2-10^3	Good
Lactococcus lactis subsp. *cremoris*	9625	10^2-10^3	Good
Streptococcus thermophilus	19258	10^2-10^3	Good

Principles of the Procedure

M17 Agar and M17 Broth contain peptones and meat derivatives as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Disodium-β-glycerophosphate buffers the medium as acid is produced from fermentation of lactose. Ascorbic acid stimulates growth of lactic streptococci. Magnesium sulfate provides essential ions for growth. Agar is the solidifying agent in M17 Agar.

Formulae

Difco™ M17 Agar

Approximate Formula* Per 950 mL

Pancreatic Digest of Casein	5.0	g
Soy Peptone	5.0	g
Beef Extract	5.0	g
Yeast Extract	2.5	g
Ascorbic Acid	0.5	g
Magnesium Sulfate	0.25	g
Disodium-β-glycerophosphate	19.0	g
Agar	11.0	g

Difco™ M17 Broth

Consists of the same ingredients without the agar.

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

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 950 mL of purified water.
   Difco™ M17 Agar – 48.25 g;
   Difco™ M17 Broth – 37.25 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. Cool to 50°C.
4. Add 50 mL sterile 10% lactose solution and mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

See appropriate references for specific procedures.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Reiter and Oram. 1962. J. Dairy Res. 29:63.
2. Lowrie and Pearce. 1971. J. Dairy Sci. Technol. 6:166.
3. Terzaghi and Sandine. 1975. Appl. Microbiol. 29:807.
4. Shankar and Davies. 1977. J. Soc. Dairy Tech. *30*:28.
5. International Dairy Federation. 1981. Identification and enumeration of microorganisms in fermented milks. Joint IDF/ISO/AOAC Group E44.
6. Richter and Vedamuthu 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ M17 Agar

COMPF ISO

Cat. No. 218571 Dehydrated – 500 g

Difco™ M17 Broth

Cat. No. 218561 Dehydrated – 500 g