

Sorbitol MacConkey Agar (NCM0167)

Intended Use

Sorbitol MacConkey Agar is used for the isolation of pathogenic *Escherichia coli* in a laboratory setting. Sorbitol MacConkey Agar is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Sorbitol MacConkey Agar is based on the formula by Rappaport and Henig. Originally developed for isolating enteropathogenic (EPEC) *serotypes*, O11 and O55, this medium is recommended for the isolation and differentiation of enterohemorrhagic *E. coli* O157:H7. Serotype O157 has been implicated in serious foodborne diseases.

MacConkey Agar W/ Sorbitol contains sorbitol instead of lactose for differentiating enteropathogenic *E. coli* serotypes; these strains are typically sorbitol negative. MacConkey Agar W/ Sorbitol is recommended for food testing.

Enzymatic Digest of Gelatin, Enzymatic Digest of Casein, and Enzymatic Digest of Animal Tissue are the nitrogen and vitamin sources in MacConkey Agar W/ Sorbitol. Sorbitol is the fermentable carbohydrate; typically enteropathogenic strains produce colorless colonies. Bile Salts Mixture and Crystal Violet are the selective agents, inhibiting Gram-positive cocci. Sodium Chloride maintains the osmotic environment, and Neutral Red is the pH indicator. Agar is the solidifying agent. The selectivity of the medium can be increased through the addition of Cefixime and tellurite.

Typical Formulation

Enzymatic Digest of Gelatin	17.0 g/L
Enzymatic Digest of Casein	1.5 g/L
Enzymatic Digest of Animal Tissue	1.5 g/L
Sorbitol	10.0 g/L
Bile Salts Mixture	1.5 g/L
Sodium Chloride	5.0 g/L
Neutral Red	0.03 g/L
Crystal Violet	0.001 g/L
Agar	13.5 g/L

Final pH: 7.1 ± 0.2 at 25°C

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

Supplement

Cefixime Tellurite Supplement X161 (1 vial per 500mL of Sorbitol MacConkey Agar)

Precaution

Refer to SDS

Preparation

1. Suspend 50 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°
5. If required add 2 vials of Cefixime Tellurite supplement X161 and mix well before dispensing.

Test Procedure

Refer to the appropriate references for specific procedures using Sorbitol MacConkey Agar.



Technical Specification Sheet



Quality Control Specifications

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

Prepared Appearance: Prepared medium is trace to slightly hazy, and medium to dark pinkish purple.

Expected Cultural Response: Cultural response on Sorbitol MacConkey Agar incubated at the appropriate atmosphere and temperature with and without X161 Cefixime Tellurite Supplement and examined for growth after 18 - 24 hours.

With supplement X161

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Recovery	Reaction
<i>Enterococcus faecalis</i> ATCC® 29212	>10 ⁵	Complete Inhibition	--
<i>Escherichia coli</i> ATCC® 25922	50-200	>50%	Pink suppressed colonies are Sorbitol positive
<i>Escherichia coli</i> ATCC® 8739	50-200	>50%	Pink suppressed colonies are Sorbitol positive
<i>Escherichia coli</i> O157:H7 ATCC® 35150	50-200	>50%	Colorless colonies are Sorbitol negative
<i>Escherichia coli</i> O157:H7 ATCC® 700728	50-200	>50%	Colorless colonies are Sorbitol negative
<i>Staphylococcus aureus</i> ATCC® 25923	>10 ⁵	Complete Inhibition	--
<i>Staphylococcus aureus</i> ATCC® 6538	>10 ⁵	Complete Inhibition	--

Without supplement X161

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Recovery	Reaction
<i>Enterococcus faecalis</i> ATCC® 29212	>10 ⁵	Complete Inhibition	--
<i>Escherichia coli</i> ATCC® 25922	50-200	>70%	Pink suppressed colonies are Sorbitol positive
<i>Escherichia coli</i> ATCC® 8739	50-200	>70%	Pink suppressed colonies are Sorbitol positive
<i>Escherichia coli</i> O157:H7 ATCC® 35150	50-200	>70%	Colorless colonies are Sorbitol negative
<i>Escherichia coli</i> O157:H7 ATCC® 700728	50-200	>70%	Colorless colonies are Sorbitol negative
<i>Staphylococcus aureus</i> ATCC® 25923	>10 ⁵	Complete Inhibition	--
<i>Staphylococcus aureus</i> ATCC® 6538	>10 ⁵	Complete Inhibition	--

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

Results

E. coli O157:H7, and other organisms that do not ferment sorbitol, are colorless on MacConkey Agar W/ Sorbitol. Sorbitol-fermenting organisms produce pink colonies. Confirmatory biochemical and serological testing should be performed on suspected colonies.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.



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Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Colonies that are sorbitol positive can revert and can be mistaken for sorbitol negative.
E. coli O157:H7 can ferment sorbitol after prolonged incubation.

Storage

Store dehydrated culture media at 2 – 30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. MacConkey, A. 1905. Lactose-fermenting bacteria in feces. *J. Hyg.* 5:333-379.
2. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
3. Mazura-Reetz, G. T. Neblett, and J. M. Galperin. 1979. MacConkey Agar: CO₂ vs. ambient incubation. *Abst. Ann. Mtg. American Society for Microbiology.* C179.