

SD-39 AGAR (6907)

Intended Use

SD-39 Agar is used in the presumptive enumeration of *Escherichia coli* O157:H7, and used with ISO-GRID and / or NEO-GRID Membrane Filtration System. SD-39 Agar conforms to AOAC Method # 997.1 for food commodities .

Product Summary and Explanation

SD-39 Agar was formulated for direct presumptive enumeration of *Escherichia coli* O157:H7, and its direct differentiation from most other *E. coli*. SD-39 Agar combines some features of Lactose Monensin Glucuronate Agar and EF-18 Agar. Formula modifications include the addition of a chromogenic substrate for detection of β -glucuronidase activity, and substitution of sorbitol for lactose or sucrose as a fermentable carbohydrate. The formula also enables direct differential enumeration of β -glucuronidase-positive *E. coli*.

SD-39 Agar is recommended for the direct, presumptive enumeration of *E. coli* O157:H7 in meats, poultry, dairy foods, infant formula, liquid eggs, mayonnaise, and apple cider^{1,2}. This medium is also used for the direct enumeration of β -glucuronidase-positive *E. coli* from foods.³

Principles of the Procedure

Enzymatic Digest of Animal Tissue and Yeast Extract are the nitrogen and vitamin sources in SD-39 Agar. Dextrose and Sorbitol are the fermentable carbohydrates. Monensin and Sodium Deoxycholate are selective agents, inhibiting Gram-positive bacteria. Novobiocin inhibits or retards the growth of some Gram-negative bacteria, including *Klebsiella* spp. Magnesium Sulfate and Sodium Glucuronate assist in repair of injured bacteria, enabling injured *E. coli* to grow in the presence of selective agents. Sodium Chloride maintains the osmotic environment and assists *E. coli* O157:H7 to grow at the specified incubation of 44.0 - 44.5°C. Phenol Red is the pH indicator dye. X-Gluc is a chromogenic substrate for β -glucuronidase. L-Lysine is present to determine whether a strain is Lysine Decarboxylase positive or negative. Agar is the solidifying agent.

Bacteria able to grow on SD-39 Agar will ferment available dextrose first. Once dextrose has been depleted, sorbitol positive bacteria will begin to ferment sorbitol, producing a local pH drop in the colony and a color change to yellow in the pH indicator, Phenol Red. Sorbitol negative bacteria that are capable of producing the lysine decarboxylase enzyme will digest L-Lysine, producing a local pH rise in the colony and a color change to pink. Bacteria that are sorbitol negative and lysine decarboxylase negative produce a mild pH drop in the colony and a color change to yellow in the pH indicator. Glucuronidase positive *E. coli* will break down X-Gluc, resulting in the production of an insoluble blue precipitate in the colony. This will combine with the color of the pH indicator dye to produce a green colony in the case of sorbitol positive or lysine negative bacteria, or a purple colony in the case of an organism that is sorbitol negative and lysine positive. A typical colony of *E. coli* O157:H7 will produce a light pink to pink colony when isolated onto this medium in the ISO-GRID / NEO-GRID Membrane Filtration System method.

Formula / Liter

Enzymatic Digest of Animal Tissue.....	5 g
Yeast Extract.....	3 g
Sodium Chloride	5 g
L-Lysine.....	10 g
Dextrose.....	2.5 g
Sorbitol.....	20 g
Magnesium Sulfate	1.5 g
Monensin	0.038 g
Sodium Deoxycholate	0.15 g
Sodium Glucuronate	0.5 g
Novobiocin	0.0075 g
Phenol Red	0.12 g
X-Gluc.....	0.05 g
Agar	15 g

Final pH: 7.2 ± 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. Suspend 63 g of the medium in one liter of purified water.
2. Heat with frequent agitation to boiling to completely dissolve the medium.
3. DO NOT AUTOCLAVE.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free-flowing, and light to medium red-beige to red yellow-beige particles.

Prepared Appearance: Prepared medium is clear to trace hazy and yellow-orange to red-orange.

Expected Cultural Response: Cultural response on SD-39 Agar, using the ISO-GRID and NEO-GRID Membrane Filtration System method. Inoculated SD-39 Agar is incubated at 44.0 - 44.5°C and examined for growth and reactions after 24 ± 2 hours.

Microorganism	Approx. Inoculum CFU	Expected Results	
		Growth	Reaction
<i>E. coli</i> O157:H7 QA-326	10 - 300	Good to excellent	Pink colonies
<i>E. coli</i> O157:H7 ATCC 35150	10 - 300	Good to excellent	Pink colonies
<i>E. coli</i> ATCC 25922	10 - 300	Good to excellent	Green colonies
<i>Klebsiella pneumonia</i> ATCC 13883	10 - 300	Partially to completely inhibited	Yellow colonies where recovered
<i>E. hermanii</i> QA-415	10 - 300	Good to excellent	Light yellow colonies

Test Procedure, ISO-GRID

1. Prepare a sample homogenate in a specified diluent.
2. Filter 1 mL of the homogenate through the pre-filter and ISO-GRID / NEO-GRID Hydrophobic Grid Membrane Filter.
3. Place the membrane filter on the surface of a pre-dried SD-39 Agar plate.
4. Incubate inverted plate for 24 ± 2 hours at 44.0 - 44.5°C. Examine membrane filter for colonies.

Results

E. coli O157:H7 is β-glucuronidase negative, sorbitol negative, lysine positive and produces pink colonies. Most other *E. coli* produce green colonies, and are β-glucuronidase positive and sorbitol positive. Occasional *E. coli* produce purple colonies, and are β-glucuronidase positive, sorbitol negative, and lysine negative.

If positive colonies are present, count the number of squares containing presumptive *E. coli* O157:H7. Convert the number of squares to the corresponding MPN and calculate the presumptive *E. coli* O157:H7 MPN, using the methods described in the ISO-GRID Methods Manual.

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.

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 nutritional variation some strains may grow poorly or fail to grow on this medium.

Packaging

SD-39 Agar **Code No.** **6907A** **500 g**

References

1. **Entis, P., and I. Lerner.** 1997. 24-Hour presumptive enumeration of *Escherichia coli* O157:H7 in food using the ISO-GRID method with SD-39 agar. J. Food Prot. **60**:883-890.
2. **Entis, P.** 1998. Direct 24-Hour presumptive enumeration of *Escherichia coli* O157:H7 in food using hydrophobic grid membrane filter, followed by serological confirmation: collaborative study. J. AOAC Int. **81**:403-418.
3. **Entis, P., and I. Lerner.** 1998. Enumeration of β -glucuronidase-positive *Escherichia coli* in foods by using the ISO-GRID method with SD-39 agar. J. Food Prot. **61**:913-916.

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.