

LM-137 AGAR BASE LM-137 SUPPLEMENT (6913)

Intended Use

LM-137 Agar Kit consists of LM-137 Agar Base (6906) and LM-137 Agar Supplement (6908). These two components are used with Egg Yolk Emulsion, 50% (7982) to prepare LM-137 Agar. LM-137 Agar is used for the presumptive enumeration of *Listeria* spp. using the ISO-GRID[®] and /or NEO-GRID[™] Membrane Filtration System in a laboratory setting. LM-137 Agar Kit is not intended for use in the diagnosis of disease or other conditions in humans.

Product Summary and Explanation

LM-137 Agar was developed for the direct presumptive enumeration of *Listeria* spp., including *Listeria monocytogenes*, from food and environmental samples. This formula was designed to encourage rapid colony development of *Listeria* spp., while retarding or inhibiting growth of Gram-negative and Gram-positive competitors. LM-137 Agar is recommended for the direct presumptive enumeration of *Listeria* spp. and *L. monocytogenes*, from meats, poultry, dairy products, frozen eggs, and environmental samples using the using the ISO-GRID and /or NEO-GRID Membrane Filtration System method.¹

Principles of the Procedure

Enzymatic Digest of Casein, Yeast Extract, Liver Peptone, and Egg Yolk emulsion are the nitrogen and vitamin sources in LM-137 Agar. Dextrose is the fermentable carbohydrate. Sodium Pyruvate is a supplementary energy source, and protects injured *Listeria* spp. from selective agents. Lithium Chloride, Acriflavin, and Di-Sodium Moxalactam (present in the LM-137 Supplement) inhibit or retard growth of many competing Gram-positive bacteria. Nalidixic Acid and Polymyxin B Sulfate (present in the LM-137 Supplement) inhibit or retard most Gram-negative bacteria. Magnesium Sulfate assists in cell wall repair and protects *Listeria* spp. from selective agents. Triphenyltetrazolium Chloride (present in the LM-137 Supplement) is a substrate for the tetrazolium reductase enzyme. Action of this enzyme results in the development of an insoluble red precipitate, producing a light pink color in *Listeria* spp. colonies. This pink color aids in the visualization and differentiation of the uninhibited competitors. Sodium Carbonate is the buffering agent. Agar is the solidifying agent.

LM-137 Agar Base

Formula / Liter

Enzymatic Digest of Casein	10 g
Yeast Extract.....	1 g
Sodium Pyruvate.....	10 g
Lithium Chloride	5 g
Magnesium Sulfate	7.4 g
Dextrose.....	1 g
Sodium Carbonate	1 g
Acriflavin	0.015 g
Liver Peptone.....	10 g
Agar	15 g

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

LM-137 Agar Supplement

Ingredients per 20 mL Bottle

Triphenyltetrazolium Chloride	0.04 g
Polymyxin B Sulfate	0.02 g
Nalidixic Acid	0.08 g
Moxalactam, Disodium	0.01 g

Egg Yolk Emulsion, 100 mL (7982)

Aseptically add 50 mL of 50% Egg Yolk Emulsion

Precautions

1. For Laboratory Use Only.
2. Harmful. Harmful if swallowed or inhaled. Harmful. May cause harm to the unborn child. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 60.4 g of the medium in 950 mL 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°C.
5. Aseptically add 50 mL of 50% Egg Yolk Emulsion (not containing tellurite) and 10 mL of rehydrated LM-137 Supplement.
6. Mix thoroughly.
7. Determine pH and aseptically adjust, if necessary, to pH 7.4 - 7.5.
8. Pour into sterile petri dishes.

Quality Control Specifications

LM-137 Agar Base

Dehydrated Appearance: Powder is homogeneous, free flowing, and light to medium tan.

Prepared Appearance: Prepared medium is clear to trace hazy and medium brown without supplements, and opaque and yellow-brown with supplements.

LM-137 Agar Supplement

Powder Appearance: Off white to very light pink.

Solution Appearance: Clear, colorless solution.

Irradiation Dosage: ≥ 25 kGY

Interfering Bioburden: Detectable microorganisms - Negative

Expected Cultural Response: Cultural response on LM-137 Agar, prepared as directed with LM-137 Supplement, using the ISO-GRID Filtration System method. Inoculated LM -137 Agar was incubated at 36 ± 1°C for 18 - 48 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reaction
<i>Escherichia coli</i> ATCC® 25922	1000	Inhibited	---
<i>Listeria monocytogenes</i> ATCC® 153134	200 - 300	Good to excellent	Pink to dark pink-orange colonies
<i>Listeria monocytogenes</i> ATCC® 19111	10 - 300	Good to excellent	Pink to dark pink-orange colonies
<i>Listeria monocytogenes</i> ATCC® 7644	10 - 300	Good to excellent	Pink to dark pink-orange colonies
<i>Listeria monocytogenes</i> ATCC® 19112	10 -300	Good to excellent	Pink to dark pink-orange colonies
<i>Staphylococcus aureus</i> ATCC® 25923	1000	Suppressed	Pale to dark orange

Test Procedure

For sample details refer to the ISO-GRID Methods Manual and NEO-GRID Protocols.

1. Prepare a sample homogenate.
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 a pre-dried prepared plate of M-137 Agar.
4. Incubate inverted plate for 18 – 48 hours at 35 - 37°C.
5. Examine membrane filter for colonies.

Results

Listeria spp. and/or *Listeria monocytogenes* are homogeneous, pink to dark pink-orange, flat, small to medium-sized colonies. Any colonies of this type are a presumptive positive for *Listeria* spp. and/or *Listeria monocytogenes*. These presumptive colonies must be confirmed using procedures detailed in the ISO-GRID Methods Manual.

If positive colonies are present, count the number of positive squares (those containing one or more colonies). The number of positive squares is converted to the corresponding most probably number (MPN) using one of 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 by keeping container tightly closed.

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

LM-137 Agar	Code No.	6906A 500 g
LM-137 Agar Supplement	Code No.	6908E 4 x 20 mL
LM-137 Agar Kit	Code No.	6913A 500 g
Egg Yolk Emulsion	Code No.	7982, 100 mL

References

1. **Entis, P. and I. Lerner.** 2000. Twenty-four hour direct presumptive enumeration of *Listeria monocytogenes* in food and environmental samples using the ISO-GRID method with LM-137 Agar. *J. Food Prot.* **63**:354-363.

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.