

PRODUCT SPECIFICATION SHEET

Kanamycin Esculin Azide Agar Base (DM762)

Intended Use

Kanamycin Esculin Azide Agar Base (DM762) is recommended for selective isolation and identification of group D *Streptococci* in foodstuff.

Product Summary and Explanation

Mossel et al^(1,2) designed Kanamycin Esculin Azide media to detect *Enterococci* in food stuffs. This medium was used by Mossel et al⁽³⁾ in the Dip Slide technique for bacteriological monitoring of foods. Kanamycin Esculin Azide Agar has been used successfully for the isolation of glycopeptide-resistant enterococci from clinical specimens and foods.^(4,5)

Principles of the Procedure

Kanamycin Esculin Azide Agar Base contains casein enzyme hydrolysate, yeast extract which provides nitrogenous and carbonaceous substances, vitamins and essential nutrients for growth of *Enterococci*. Kanamycin sulphate and Sodium azide are the selective inhibitory components. Esculin and Ferric ammonium citrate together form indicator system to detect esculin - hydrolysing Streptococci forming black zones around the colonies.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	20.00
Yeast extract	5.00
Sodium chloride	5.00
Sodium citrate	1.00
Esculin	1.00
Ferric ammonium citrate	0.50
Sodium azide	0.15
Agar	10.00
Final pH : 7.0 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

Directions

1. Suspend 21.32 grams of the medium in 500 ml of distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 45-50°C and aseptically add rehydrated contents of one vial of Kanamycin Sulphate Selective Supplement (MS039).
5. Mix well before pouring into sterile petri plates.

Quality Control Specifications

Dehydrated Appearance	Light yellow to light brown coloured homogeneous free flowing powder
Prepared Medium	Medium amber coloured clear to slightly opalescent gel with purplish tinge forms in petri plates
Reaction of 4.26% Solution	pH : 7.0 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.0% Agar gel



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Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C or 42°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
1.	<i>Enterococcus bovis</i> ATCC 27960	50 -100	good-luxuriant	≥50%	Positive reaction, blackening of medium around the colony
2.	<i>Enterococcus faecium</i> ATCC 19434	50 -100	good-luxuriant	≥50%	Positive reaction, blackening of medium around the colony
3.	<i>Escherichia coli</i> ATCC 25922	50 -100	inhibited	0%	--

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

Test Procedure

1. Prepare tubes of sterilised Tryptone Water (DM279) in 9ml volumes. Chill to 0-5°C by storing in a refrigerator for 18 hours prior to use.
2. Add 1g or 1ml of the thoroughly mixed food sample to a tube containing 9ml of pre-chilled diluent (10⁻¹ dilution). Shake well for 30 seconds.
3. Sample 1ml of the contents, within 30 seconds after mixing, into a fresh tube of diluent. Continue the process using fresh sterile pipettes until a dilution is reached which will produce 100 colonies per 1ml.
4. Store the decimal dilutions in the refrigerator and examine within 3 hours of their preparation.
5. Streak onto plates of Kanamycin Esculin Azide Agar (DM762) and after incubation confirmatory tests are carried out.⁽⁶⁾
6. Refer to appropriate references for standard test procedures.

Results

Positive results for enterococci observed as colonies surrounded by black haloes are grown. Refer to appropriate references and test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 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 the 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.

Limitations of the Procedure

1. There is no universal medium which will isolate all strains of *enterococci*.⁽⁷⁾
2. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
3. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Kanamycin Esculin Azide Agar Base

Product Code : DM762

Available Pack sizes : 100gm





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References

1. Mossel D.A.A., Bijker, P.G.H. and Eelderink I., 1978, Arch. Lebensmittel - Hyg., 29:121.
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3. Mossel D.A.A., et al, 1976, Lab. Practice, 25:393.
4. Mossel D.A.A., Harrenwijn G.A. and Elzebroek B.J.M., 1973, UNICEF Geneva.
5. Chadwick P.R., Brown D.F.J., Wilcox M.H. et al (1997) *Clin. Microbiol. Inf.* 3. 559-563.
6. Van den Braak N., Van Belkum A., Van Keulen M. et al. (1998) *J. Clin. Microbiol.* 36. 1927-1932.
7. Reuter E., 1985, Inter. J. Food Microbiol., 2:103.

Further Information

For further information please contact your local MICROMASTER Representative.



MICROMASTER LABORATORIES PRIVATE LIMITED

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Unit 38/39, Kalpataru Industrial Estate,
Off G.B. Road, Near 'R-Mall' , Thane (W) - 400607. M.S. INDIA.
Ph: +91-22-25895505, 4760, 4681. Cell: 9320126789.

Email: micromaster@micromasterlab.com
sales@micromasterlab.com

Prepared By	Checked By	Approved By
Microbiologist	Head Quality Control	Head Quality Assurance

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