



PRODUCT SPECIFICATION SHEET

Sellers Differential Agar (DM469)

Intended Use

Sellers Differential Agar (DM469) is recommended for differentiation and identification of gram-negative non-fermentative bacilli especially *Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus*.

Product Summary and Explanation

In 1964, Sellers⁽¹⁾ formulated a medium Sellers Differential Agar, for differentiation and identification of non-fermentative gram-negative bacilli especially *Pseudomonas aeruginosa*, *Acinetobacter calcoaceticus* and *Alcaligenes faecalis*. The medium is complex with differentiation ability based on oxidation of dextrose, fluorescence, production of nitrogen and pH changes.

Principles of the Procedure

Sellers Differential Agar contains yeast extract and peptic digest of animal tissue which are the sources of carbon and nitrogen compounds as well as vitamins and minerals. The dextrose added prior to inoculation diffuses into the medium during incubation period. *P. aeruginosa* exhibits acid reaction from dextrose. However, the reaction is masked by deamination of arginine and high peptone concentration. D-Mannitol and magnesium sulphate stimulate fluorescence while nitrogen gas production is stimulated by dipotassium phosphate. Sodium nitrate and nitrite serve as substrates for the production of nitrogen gas for denitrifying bacteria. Phenol red and bromothymol blue are the pH indicators.

Formula / Liter

Ingredients	Gms / Liter
Yeast extract	1.00
Peptic digest of animal tissue	20.00
L-Arginine	1.00
D-Mannitol	2.00
Sodium chloride	2.00
Sodium nitrate	1.00
Sodium nitrite	0.35
Magnesium sulphate	1.50
Dipotassium phosphate	1.00
Bromo thymol blue	0.04
Phenol red	0.008
Agar	15.00
Final pH: 6.7 ± 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.

Directions

1. Suspend 44.89 grams of medium in one liter of distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Dispense in test tubes.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Cool the tubed medium in slanted position.





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6. Just before inoculation add 0.15 ml or 2 drops of 50% sterile dextrose solution to each slant by letting it run down the side of the tube opposite the slant.

Quality Control Specifications

Dehydrated Appearance	Light yellow to pink homogeneous free flowing powder
Prepared Medium	Green coloured clear to slightly opalescent gel forms in tubes as slants with a butt
Reaction of 4.5% Solution	pH : 6.7 ± 0.2 at $25^\circ C$
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed after an incubation at $35 - 37^\circ C$ for 18 - 24 hours.

Sr. No.	Organisms	Results to be achieved					
		Inoculum (CFU)	Growth	Slant	Butt	Band	Fluorescence (under uv)
1.	<i>Acinetobacter baumannii</i> ATCC 19606	50 - 100	good	blue	green	yellow	negative
2.	<i>Alcaligenes faecalis</i> ATCC 8750	50 - 100	good	blue	blue-green	none	positive
3.	<i>Pseudomonas aeruginosa</i> ATCC 27853	50 - 100	good	blue-green	blue-green	blue	positive

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

Test Procedure

1. To inoculate, carefully touch only the center of an isolated colony with a sterile needle, stab into the medium in the butt of the tube, and then streak back and forth along the surface of the slant.
2. Refer to appropriate references for standard test procedures.

Results

1. Oxidation of dextrose by the organisms is readily visible as a yellow band at the slant-butt junction.
2. Arginine dihydrolase positive reaction is indicated by the formation of blue colour.
3. Most of *Acinetobacter* species produce a yellow band due to glucose oxidation. This band may disappear after 24 hours.

Storage

Store the sealed bottle containing the dehydrated medium at $10 - 30^\circ C$. 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. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.





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Packaging

Product Name : Sellers Differential Agar

Product Code : DM469

Available Pack sizes : 500gm

References

1. Sellers W., 1964, J. Bacteriol., 87:46.

Further Information

For further information please contact your local MICROMASTER Representative.



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