



PRODUCT SPECIFICATION SHEET

R-3A Agar (DM555)

Intended Use

R-3A Agar (DM555) is recommended for sub-culturing microorganisms recovered on less nutritive R-2A Agar from potable water samples.

Product Summary and Explanation

Reasoner and Geldreich⁽¹⁾ formulated R-2A Agar which is recommended by APHA^(2, 3) for estimating the heterotrophic plate count by the pour plate, spread plate or membrane filter procedure. Stressed or injured organisms during water treatment are unable to grow on high nutrient media, since the faster growing organisms outgrow the former.⁽⁴⁾ For that reason the use of a low nutrient medium like R-2A Agar incubated for longer incubation periods allows these stressed organisms to grow well.

R-3A Agar is slightly more nutritious than R-2A Agar and is used for sub-culturing the isolates obtained on the less nutritive R-2A Agar.⁽⁵⁾

Principles of the Procedure

R-3A Agar contains biopeptone, casein acid hydrolysate and yeast extract which provides nitrogen, vitamins, amino acids, carbon and minerals. Dextrose serves as an energy and carbon source. Soluble starch aids in the recovery of injured organisms by absorbing toxic metabolic byproducts while sodium pyruvate increases the recovery of stressed cells. Magnesium sulphate is a source of divalent cations and sulphate. Dipotassium phosphate is used to balance the pH of the medium.

Formula / Liter

Ingredients	Gms / Liter
Casein acid hydrolysate	1.00
Yeast extract	1.00
Biopeptone	1.00
Dextrose	1.00
Starch, soluble	1.00
Dipotassium phosphate	0.60
Magnesium sulphate	0.048
Sodium pyruvate	0.60
Agar	15.00
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 only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 10 grams of medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.





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3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. DO NOT OVERHEAT.
5. Mix well and pour into sterile petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light yellow coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 2.13% Solution	pH : 7.2 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed *by using standard ATCC cultures after an incubation at 35-37°C for 24-72 hours. (*-In case of water samples from fields it is suggested to incubate further for upto 7 days to ascertain the absence of organisms)

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Recovery
1.	<i>Candida albicans ATCC 10231</i>	50 - 100	good-luxuriant	≥50%
2.	<i>Enterococcus faecalis ATCC 29212</i>	50 - 100	good-luxuriant	≥50%
3.	<i>Escherichia coli ATCC 25922</i>	50 - 100	good-luxuriant	≥50%
4.	<i>Salmonella Enteritidis ATCC 13076</i>	50 - 100	good-luxuriant	≥50%
5.	<i>Salmonella Typhi ATCC 6539</i>	50 - 100	good-luxuriant	≥50%

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

Test Procedure

Refer to appropriate references for standard test procedures.

Results

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. 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 : R-3A Agar

Product Code : DM555

Available Pack sizes : 500gm

References

1. Reasoner D. J. and Geldreich E. E., 1985, Appl. Environ. Microbiol., 49:1.
2. Clesceri L. S., Greenberg A. E. and Eaton A. D., (Ed.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th Ed., American Public Health Association, Washington, D.C.
3. Downes F. P. and Ito K., (Eds.), Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
4. Collins V. J. and Willoughby J. G., 1962, Arch. Microbiol., 43:294.
5. Atlas R. M., 2004, Handbook of Microbiological Media, Lawrence C. Parks, (Ed.), 3rd Edition, CRC Press.

Further Information

For further information please contact your local MICROMASTER Representative.



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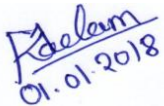


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