

PRODUCT SPECIFICATION SHEET

Tryptose Cycloserine Azide Agar Base (DM1003)

Intended Use

Tryptose Cycloserine Azide Agar Base (DM1003) is recommended for enumeration of sulphite reducing anaerobes essentially *Clostridia*.

Product Summary and Explanation

Many media have been developed to enumerate *Clostridium* from foods. Hauschild and Hilsheimer originally formulated Tryptose Cycloserine Azide Agar Base for enumeration of sulphite reducing anaerobes essentially *Clostridia*.⁽¹⁾ This medium was later modified by decreasing the concentration of D-cycloserine, sulphite and iron and by the addition of sodium azide.^(2,3) This medium makes use of the selective inhibitory properties of D-cycloserine and an indicator system involving sulphite and iron. *C. perfringens* and related species which reduces the sulphite and form black colonies due to the production of ferrous sulphide and growth of non-mesophilic organisms are suppressed.⁽⁴⁾

Principles of the Procedure

Tryptose Cycloserine Azide Agar Base contains tryptose, papaic digest of soyabean meal, meat extract and yeast extract which provides essential nitrogenous compounds and vitamins needed for the growth of anaerobes. Glucose serves as carbon and energy source. Disodium disulphite is reduced to hydrogen sulphide which combines with ferric ions of ferric salts to produce the insoluble black precipitate of ferrous sulphide. D-Cycloserine (MS056) and sodium azide inhibit a number of organisms including *Bacillus* species, enteric bacilli, *Proteus*, *Pseudomonas* and most of the cocci. Some anaerobes reduce sulphite to hydrogen sulphide (H₂S) due to presence of ferric ammonium citrate, which is indicated by blackening of the colonies.

Formula / Liter

Ingredients	Gms / Liter
Tryptose	15.00
Papaic digest of soyabean meal	5.00
Meat extract	5.00
Yeast extract	5.00
Glucose	2.00
Disodium disulphite	0.50
Ferric ammonium citrate	0.50
Sodium azide	0.05
Agar	14.00
Final pH: 7.4 ± 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 23.52 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 50°C and aseptically add 1.5 ml rehydrated contents of 1 vial of Perfringens T.S.C. Supplement (MS056) for 500 ml medium.
5. Mix well and pour into sterile Petri plates.

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Quality Control Specifications

Dehydrated Appearance	Cream to brownish yellow homogeneous free flowing powder
Prepared Medium	Yellow to amber coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 4.7% Solution	pH : 7.4 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.4% Agar gel

Expected Cultural Response: Cultural response observed after an incubation at 35-37°C for 18-24 hours with added Perfringes T.S.C. Supplement (MS056).

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Colour of colony
1.	<i>Clostridium perfringens</i> ATCC 12924	50 -100	good-luxuriant	≥50%	black
2.	<i>Clostridium sporogenes</i> ATCC 11437	50 -100	good-luxuriant	≥50%	black
3.	<i>Escherichia coli</i> ATCC 25922	≥10 ³	inhibited	0%	--
4.	<i>Staphylococcus aureus</i> ATCC 25923	≥10 ³	inhibited	0%	--

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

Test Procedure

Refer appropriate references for standard test procedures.

Results

Refer 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.

Packaging

Product Name : Tryptose Cycloserine Azide Agar Base

Product Code : DM1003

Available Pack sizes : 500gm

References

1. Hauschild A. H. W. and Hilsheimer R., 1974, Appl. Microbiol., 27, 521-527.
2. Eisgruber H., 1986, Vet Med. Diss. FU Berlin.
3. Eisgruber H. and Reuter G., 1991, Arch. Lebensmittelhyg, 42,125-129.
4. Corry J. E. L., Curtis G. D. W. and Baird R. M., 1995, Culture Media for Food Microbiology, Vol. 34, ELSEVIER, Amsterdam.



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Further Information

For further information please contact your local MICROMASTER Representative.



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