



PRODUCT SPECIFICATION SHEET

Xylose-Lysine Deoxycholate Agar (DM297B)

Intended Use

Xylose-Lysine Deoxycholate Agar (DM297B) is recommended for selective isolation and enumeration of *Salmonella typhi* and other *Salmonella* species from pharmaceutical products using microbial limit testing in compliance with BP.

Product Summary and Explanation

The Enterobacteriaceae is a large family of Gram-negative bacteria that includes, along with many harmless symbionts, many of the more familiar pathogens, such as *Salmonella*, *Escherichia coli*, *Yersinia pestis*, *Klebsiella* and *Shigella*. Other disease-causing bacteria in this family include *Proteus*, *Enterobacter*, *Serratia*, and *Citrobacter*. They normally inhabit the intestines of humans and animals.⁽¹⁾ Human *Salmonella* infections are most commonly caused by ingestion of food, water or milk, contaminated by human or animal excreta.⁽²⁾ A wide variety of media have been developed to aid in the selective isolation and differentiation of enteric pathogens. Due to the large numbers of different microbial species and strains with varying nutritional requirements and chemical resistance patterns, investigators have developed various formulae to meet general as well as specific needs relative to isolation and identification of the microorganisms. Xylose Lysine Deoxycholate Agar is a selective as well as differential medium formulated by Taylor⁽³⁻⁷⁾ for the isolation and identification of enteric pathogens especially Shigellae from stool samples. This medium is also employed for pharmaceutical testing and non-sterile product testing for the detection (or absence) of *Salmonella* after enrichment in Rappaport Vassiliadis Salmonella Enrichment Broth (DM1405B). This medium is prepared according to BP⁽⁹⁾ and is in accordance with the harmonized method of USP/BP/EP/JP.⁽⁸⁻¹¹⁾

Principles of the Procedure

Xylose-Lysine Deoxycholate Agar contains yeast extract which is a source of nitrogen, carbon, and vitamins required for organism growth. Deoxycholate, ferric ammonium citrate and sodium thiosulphate are selective agents that inhibit gram-positive microorganisms. Xylose, lactose, and sucrose are fermentable carbohydrate sources. Almost all the enteric bacteria except Shigellae, ferment xylose fermented which enables the differentiation of Shigellae from Salmonellae. Salmonellae metabolize the xylose and decarboxylate lysine and thus changing the pH to alkaline and mimic Shigellae reaction. Lactose and sucrose are added in excess to produce acid and hence non-pathogenic H₂S producers do not decarboxylate lysine thereby preventing Shigellae reaction by lysine positive coliforms. Sodium thiosulphate prevents the discoloration of these compounds during storage by reactivating sulphur containing compounds. It also forms the substrate for enzyme thiosulphate reductase, which breaks it to form H₂S. Thiosulphate and ferric ammonium citrate forms H₂S indicator system. Sodium thiosulphate is also inactivator of halogens, mercurial and aldehyde and can minimize its toxicity in the testing sample, if any during microbial limit tests. Sodium chloride maintains the osmotic equilibrium in this medium. Phenol red is the pH indicator.

Formula / Liter

Ingredients	Gms / Liter
Xylose	3.50
L-Lysine	5.00
Lactose monohydrate	7.50
Sucrose	7.50
Sodium chloride	5.00
Yeast extract	3.00
Phenol red	0.08
Sodium deoxycholate	2.50





PRODUCT SPECIFICATION SHEET

Sodium thiosulphate	6.80
Ferric ammonium citrate	0.80
Agar	13.50
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.

Directions

1. Suspend 54.8 g (the equivalent weight of dehydrated medium per litre) in one liter of purified water.
2. Heat with frequent agitation until the medium boils.
3. DO NOT OVERHEAT OR AUTOCLAVE.
4. Transfer immediately to a water bath at 50°C. After cooling, pour into sterile Petri plates.
5. It is advisable not to prepare large volumes, which will require prolonged heating and may produce precipitate.

Quality Control Specifications

Dehydrated Appearance	Light yellow to pink homogeneous free flowing powder
Prepared Medium	Red coloured clear to slightly opalescent gel forms in Petri plates
Reaction of % Solution	Not Applicable
Gel Strength	Firm, comparable with 1.35 % Agar gel

Cultural Response

Growth Promotion is carried out in accordance with the harmonized method of BP. Cultural response was observed after an incubation at 30-35°C for specified time. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Growth promoting properties

Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu(at 30-35°C for <=18 hours).

Indicative properties

Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating <=100cfu (at 30-35°C for 18-72 hours).

Inhibitory properties

No growth of the test microorganism occurs for the specified temp for not less than longest period of time specified inoculating >=100cfu (at 30-35°C for >= 72 hours).

Expected Cultural Response: Cultural characteristics observed after incubation at 30-35 °C for 18-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Sr. No.	Organisms	Results to be achieved					
		Inoculum	Growth	Observed	Recovery	Colour of	Incubation





PRODUCT SPECIFICATION SHEET

		(CFU)		Lot value(CFU)		Colony	period
	Growth Promoting + Indicative						
1.	<i>Salmonella Typhimurium</i> ATCC 14028	50 -100	luxuriant	25 -100	≥50 %	red with black centres	18 -72 hrs
2.	<i>Salmonella Abony</i> NCTC 6017	50 -100	good-luxuriant	25 -100	≥50 %	red with black centres	18 -72 hrs
	Additional Microbiological testing						
3.	<i>Escherichia coli</i> ATCC 8739	50-100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
4.	<i>Escherichia coli</i> ATCC 25922	50-100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
		Results to be achieved					
Sr. No.	Organisms	Inoculum (CFU)	Growth	Observed Lot value(CFU)	Recovery	Colour of Colony	Incubation period
5.	<i>Escherichia coli</i> NCTC 9002	50-100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
6.	<i>Proteus vulgaris</i> ATCC 13315	50 -100	good-luxuriant	25 -100	≥50 %	grey with black centres	18 -72 hrs
7.	<i>Salmonella Paratyphi A</i> ATCC 9150	50 -100	good-luxuriant	25 -100	≥50 %	red	18 -72 hrs
8.	<i>Salmonella Paratyphi B</i> ATCC 8759	50 -100	good-luxuriant	25 -100	≥50 %	red with black centers	18 -72 hrs
9.	<i>Salmonella Enteritidis</i> ATCC 13076	50 -100	good-luxuriant	25 -100	≥50 %	red with black centers	18 -72 hrs
10.	<i>Salmonella Typhi</i> ATCC 6539	50 -100	good-luxuriant	25 -100	≥50 %	red with black centers	18 -72 hrs
11.	<i>Shigella dysenteriae</i> ATCC 13313	50 -100	good-luxuriant	25 -100	≥50 %	red	18 -72 hrs
12.	<i>Shigella flexneri</i> ATCC 12002	50 -100	fair-good	15 -40	30 -40 %	red	18 -72 hrs
13.	<i>Shigella sonnei</i> ATCC 25931	50 -100	fair-good	15 -40	30 -40 %	red	18 -72 hrs
14.	<i>Enterobacter aerogenes</i> ATCC 13048	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
15.	<i>Enterobacter cloacae</i> ATCC 13047	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
16.	<i>Staphylococcus aureus</i> ATCC 25923	≥10 ³	inhibited	0	0%	--	≥72 hrs
17.	<i>Staphylococcus aureus</i> ATCC 6538	≥10 ³	inhibited	0	0%	--	≥72 hrs





PRODUCT SPECIFICATION SHEET

18.	<i>Enterococcus faecalis</i> ATCC 29212	$\geq 10^3$	inhibited	0	0%	--	≥ 72 hrs
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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

1. Degradation of fermentable sugars proceed concurrently and generates acids, which cause pH indicator to give various shades of colour, causing a colour change in the colonies and in the medium from red to yellow on prolonged incubation.
2. Hydrogen sulfide production results in colonies with black centers under alkaline conditions, which can be inhibited by acid production by carbohydrate fermentation. Alkaline condition causes the colour of the medium to change back to red.
3. This medium is an ideal medium for screening samples containing mixed flora of enteric pathogens as recovery of Salmonellae and Shigellae is not conspicuous by even profuse growth of other species.^(12,13)

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. Red, false-positive colonies may occur with some *Proteus* and *Pseudomonas* species.
2. Incubation in excess of 48 hours may lead to false-positive results.
3. *S. Paratyphi A*, *S. Choleraesuis*, *S. pullorum* and *S. gallinarum* may form red colonies without black centers, thus resembling *Shigella* species.
4. Some *Proteus* strains will give black-centered colonies on XLD Agar.
5. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Xylose-Lysine Deoxycholate Agar

Product Code : DM297B

Available Pack sizes : 100gm / 500gm

References

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PRODUCT SPECIFICATION SHEET

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Further Information

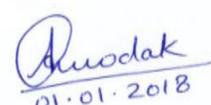
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