Technical Data Sheet

Xylose Lysine Deoxycholate (XLD) Agar acc. harm. EP/USP/JP

Ordering number: 1.05290.0500

Xylose lysine deoxycholate (XLD) agar was proposed by Taylor (1965), Taylor and Harris (1967) and Taylor and Schelhart (1967, 1968) for the isolation and differentiation of pathogenic *Enterobacteriaceae*, especially *of Shigella* and *Salmonella* species.

This medium complies with the specifications given by the harmonized methods of EP, USP, JP for Microbial Examination of Non-sterile Products: Tests for Specified Microorganisms.

Mode of Action

Degradation of xylose, lactose and sucrose to acid causes phenol red to change its color to yellow. Production of hydrogen sulfide is indicated by thiosulfate and iron(III) salt, which react to form a precipitate of black iron sulfide in the colonies.

Bacteria which decarboxylate lysine to cadaverine can be recognized by the appearance of a purple coloration around the colonies due to an increase in pH.

These reactions can proceed simultaneously or successively, this may cause the pH indicator to exhibit various shades of color or it may change its color from yellow to red on prolonged incubation. The culture medium is weakly inhibitory.

Typical Composition

Yeast Extract	3 g/l
NaCl	5 g/l
D(+)-Xylose	3.5 g/l
Lactose Monohydrate	7.5 g/l
Sucrose	7.5 g/l
L(+)-Lysine	5 g/l
Sodium Deoxycholate	2.5 g/l
$Na_2S_2O_3$	6.8 g/l
Ammonium Iron(III) Citrate	0.8 g/l
Phenol Red	0.08 g/l
Agar-Agar	13.5 g/l

Preparation

1. Weigh out 55.2 g of XLD Agar.
2. Add 50 ml of demineralized water to a flask.
3. Transfer 55.2 g of XLD Agar gently to flask with swirling.
4. Mix thoroughly, add remaining 950 ml demineralized water, until completely suspended. Check for lumps. If present repeat mixing.
5. Heat to boiling to dissolve completely (variously shaking).
6. Immediately cool the medium to about 47-50 ℃ in a waterbath set at this temperature. Agitate flask to cool rapidly.
7. Pour plates.
8. Dry plates and check for sterility prior to use.

Note: Preparation of large volumes, overheating and prolonged storage in water bath (47-50 ℃) should be avoided.

Do not autoclave.

The appearance of the plates is clear and red.

The pH value at 25 ℃ is in the range of 7.2-7.6.

Experimental Procedure and Evaluation

Inoculate by spreading the material thinly on the surface of the plates.

Incubation: For *Salmonella* up to 18 h at 30-35 ℃, for *Escherichia coli* up to 48 h at 30-35 ℃ aerobically.

Typical colonies of *Salmonella* will show a black center and a slightly red colored translucent zone due to the indicator color change. H_2S-negative *Salmonella* (e.g. *Salmonella* Paratyphi A) will grow pink with a dark pink center. Beside XLD Agar a second selective medium free of choice has to be used. The degradation of xylose causes acidification combined with a color change of the medium to yellow.

Strains containing lysine-decarboxylase will build up cadaverine (1,5-diaminopentane) from L(+)-lysine which will cause alkalization of the medium indicated by a red violet color of the medium. The activity of lysine-decarboxylase will neutralize acids of xylose degradation and the medium will show a red violet color.

Lactose and sucrose is added in order to differentiate lysine-decarboxylase-positive coliform bacteria from *Salmonella*. The formation of acids cannot be neutralized by cadaverine and the medium will be colored yellow. In addition, deoxycholate will inhibit the growth of coliform bacteria.

Thiosulfate and iron(III) salt will react with hydrogen sulfide to black iron sulphate. Hydrogen sulfide producing bacteria will show a black precipitation center within their colonies.

Sodium thiosulfate is reduced by *Salmonella* and other sulfate reducing bacteria to H_2S, which will then reduce ferric ammonium citrate to a black colored iron sulfide. This reaction works better under alkaline conditions, wherefore H_2S-building bacteria which form acids from lactose and/or sucrose may not show a strong black center within their colonies (e.g. *Citrobacter spp.* and *Proteus spp*.).

Salmonella Typhi will show frequently yellow-orange or pink colored colonies.

The table below shows some typical appearances of colonies on XLD Agar:

Appearance of Colonies	Microorganisms
Colonies have the same colour as the culture medium, sometimes with a black centre	*Salmonella*
Orange, slightly opaque	*Salmonella typhosa* (xylose-positive strains)
Colonies have the same colour as the culture medium, translucent	*Shigella, Providencia, Pseudomonas*
Yellow, surrounded by yellow zones, opaque with precipitation zones	*Escherichia coli, Enterobacter, Aeromonas*
Yellow, surrounded by yellow zones, opaque, mucoid with precipitation zones	*Klebsiella*
Yellow, surrounded by yellow zones, opaque, sometimes with a black centre	*Citrobacter* (lactose-positive strains)
Yellow, surrounded by yellow zones, opaque	*Serratia, Hafnia*
Yellow, surrounded by yellow zones, translucent, black centre	*Proteus vulgaris*, most *Proteus mirabilis*

Storage

The product can be used for sampling until the expiry date if stored upright, protected from light and properly sealed at +15 ℃ to +25 ℃.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 ℃ to +25 ℃.

Disposal

Please mind the respective regulations for the disposal of used culture medium (e.g. autoclave for 20 min at 121 ℃, disinfect, incinerate etc.).

Quality Control

Control Strains	ATCC #	Inoculum CFU	Incubation	Expected Results
Escherichia coli	8739	-	48 h at 30-35 ℃	None to very good growth, yellow colonies, yellow medium color, precipitation
Salmonella Typhimurium	14028	10-100	18 h at 30-35 ℃	Recovery ≥ 50 %, red colonies with black centre, medium unchanged
Salmonella abony	6017 (NCTC #)	10-100	18 h at 30-35 ℃	Recovery ≥ 50 %, red colonies with black centre, medium unchanged

Please refer to the actual batch related Certificate of Analysis.

Literature

European Directorate for the Quality of Medicines and Healthcare. (2014): The European Pharmacopoeia. 8th Ed. Chapter 2.6.13 Microbiological examination of non-sterile products: Test for specified products. Strasbourg, France.

Japanese Ministry of Health, Labour and Welfare. (2011): The Japanese Pharmacopoeia. 16th Ed. Chapter 4.05 Microbial Limit Test II. Microbiological examination of non-sterile products: Test for specified products. Japanese Ministry of Health, Labour and Welfare. Tokyo, Japan.

Taylor, W.J. (1965). Isolation of *Shigellae*. Am. J. Clin. Path. **44**: 471-479.

Taylor, W.J., and Harris, B. (1967). Isolation of *Shigellae*. III. Comparison of new and traditional media with stool specimens. Amer. J. Clin. Pathol. **48**: 350-355.

United States Pharmacopoeia 38 NF 33 (2015): <62> Microbiological examination of non-sterile products: Tests for specified microorganisms.

Ordering Information

Product	Cat. No.	Pack size	Other pack sizes available
Xylose Lysine Deoxycholate (XLD) Agar	1.05290.0500	500 g	
Tryptic Soy Broth	1.05459.0500	500 g	5 kg, 25 kg
Rappaport Vassiliadis Salmonella Enrichment Broth	1.07666.0500	500 g	

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