

## Technical Data Sheet

# Xylose Lysine Deoxycholate (XLD) Agar – LI acc. EP/USP

Ordering number: 1.46073.0020 / 1.46073.0120

Xylose Lysine Deoxycholate (XLD) Agar – LI is a selective culture medium for isolation of *Salmonella* and *Shigella* in pharmaceuticals, foodstuff and clinical materials.

Ten settle plates each with a diameter of 90 mm are single-bagged in transparent, hydrogen peroxide impermeable sleeves (non-irradiated). The sleeves consist of polypropylene with a barrier of PE-EVOH-PE.

The formulation of the basic medium is prepared according to the recommendations of the current European and United States Pharmacopoeia (EP, 2.6.12. and USP, 61).

### 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
L-Lysine	5 g/l
Sucrose (Saccharose)	7.5 g/l
Xylose	3.5 g/l
Lactose Monohydrate	7.5 g/l
Sodium Deoxycholate	2.5 g/l
Ammonium Iron(III) Citrate	0.8 g/l
Sodium Thiosulfate	6.8 g/l
NaCl	5 g/l
Phenol Red	80 mg/l
Agar	13.5 g/l

The appearance of the medium is clear and red, possibly with white crystals. The pH value is in the range of 7.2-7.6. The medium can be adjusted and/or supplemented according to the performance criteria required.

### Application and Interpretation

Each plate is provided with a label including a data matrix code for paperless plate identification. The code consists of a two-dimensional 20-digit serial number, which harbors the following information:

Digits 1-3: here code 796 (corresponds to article 146073); digits 4-9: lot number; digits 10-14: batch specific individual number; digits 15-20: expiration date (YY/MM/DD).

Please check each agar plate before using it on sterility and pay attention to aseptic handling in order to avoid false positive results.

According to the harmonized chapters of EP and USP the absence test for *Salmonella* is prepared as follows: At first 10 g of the product are cultured within Tryptic Soy Broth (e.g. article number 146334) for 18-24 hours at 30-35 °C. From this culture 0.1 ml is retransferred into 10 ml Rappaport Vassiliadis Broth (article number 146181) and incubated for 18-24 hours at 30-35 °C. At least a subculture from Rappaport Vassiliadis Broth is prepared on XLD-Agar and incubated for 18-24 hours at 30-35 °C. Well developed, red colonies, with or without black centers may indicate the presence of *Salmonellae*.

According to the recommendations of EN ISO 6579 for foodstuffs a 1:10 dilution of the sample is prepared within Buffered Peptone Water (e.g. article number 146403) and incubated for 16-20 h at 36-38 °C.

From this non-selective pre-enrichment 0.1 ml is transferred into 10 ml Rappaport Vassiliadis Medium (article number 146181) and incubated for 21-27 h at 40.5-42.5 °C and 1 ml is transferred in 10 ml Tetrathionate Broth (MKTTn, article number 146221) and incubated for 21-27 h at 36-38 °C. From the selective enrichment cultures each a subculture on XLD Agar is prepared and incubated for 21-27 h at 36-38 °C. Typical colonies of *Salmonella* will show a black center and a slightly red colored translucent zone due to the indicator color change. H<sub>2</sub>S-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(II) 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<sub>2</sub>S, which will then reduce ferric ammonium citrate to a black colored iron sulfide. This reaction works better under alkaline conditions, wherefore H<sub>2</sub>S-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.

**Note:** The specificity of the medium is reduced if incubation times exceed 24 hours.



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The table below will show some typical reactions of the XLD Agar:

Colony Characteristics	Indicator Reactions	Xyl	Lys	Lac/Suc	H <sub>2</sub> S	Possible Microorganisms
red, translucent colonies	no color change, no formation of iron sulfide	-	-	-	-	<i>Shigella</i> , <i>Providencia</i> <i>Pseudomonas spp.</i>
colorless to pale pink colonies, medium red to red violet, colonies mostly with black center	alkalization, color change to red violet, mostly formation of iron sulfide	+	+	-	+/-	<i>Salmonella</i> <i>Edwardsiella spp.</i>
colorless to pale pink colonies, medium red to red violet, colonies without black center	alkalization, color change to red violet, no formation of iron sulfide	+	+	-	-	<i>Salmonella</i> Paratyphi A, H <sub>2</sub> S-negative <i>Salmonella</i>
yellow, opaque colonies on yellow medium (weak black center <i>Citrobacter</i> and possibly at <i>Proteus</i> )	acidification, color change to yellow, no formation of iron sulfide (possible is a slight formation of iron sulfide for <i>Citrobacter</i> and <i>Proteus</i> )	+/-	+/-	+	+/-	<i>E. coli</i> <i>Enterobacter</i> <i>Klebsiella</i> <i>Serratia</i> <i>Citrobacter</i> <i>Proteus spp.</i>

Xyl = Xylose degradation; Lys = Lysine-decarboxylase, Lac/Suc = Lactose and/or Sucrose degradation; according to MacFaddin

According to EP and USP suspect colonies have to be identified using suitable methods. The EN ISO 6579 contains detailed information concerning the confirmation of suspect colonies.

Besides XLD Agar also the chromogenic *Salmonella* elective agar according to Rambach (article number 146719) as well as further selective media like BPLS Agar (article number 146046), LEIFSON Agar (article number 102896), Hektoen Enteric Agar (article number 111681), Modified Semi-Solid Rappaport-Vassiliadis (MSRV) Medium (article number 146622) and Salmonella Shigella (SS) Agar (article number 107667) are available.

For biochemical examination of suspect colonies for example Triple Sugar Iron Medium (article number 103915) or Tryptophan Broth (article number 146731) for indole testing are available.

### Storage and Shelf Life

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

Condensation can be prevented by avoiding quick temperature shifts and mechanical stress.

The testing procedures as described on the CoA can be started up to the expiry date printed on the label.

### Disposal

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



## Quality Control

Control Strains	ATCC #	Inoculum CFU	Incubation	Expected Results
<i>Salmonella</i> Typhimurium	14028	10-100	16-18 h at 30-35 °C	Recovery 50-200 %
			18-24 h at 30-35 °C	Good growth; pale-pink colonies with large black center; nutrient medium unchanged
<i>Salmonella</i> Abony	6017 (NCTC #)	10-100	16-18 h at 30-35 °C	Recovery 50-200 %
			18-24 h at 30-35 °C	Good growth; pale-pink colonies with large black center; nutrient medium unchanged
<i>Escherichia coli</i>	8739	10-100	18-24 h at 35-37 °C	Recovery ≤ 5 %
<i>Staphylococcus aureus</i>	6538	10,000-100,000	18-24 h at 35-37 °C	No growth
<i>Enterococcus faecalis</i>	19433	10,000-100,000	18-24 h at 35-37 °C	No growth

Please refer to the actual batch related Certificate of Analysis.

## Literature

European Pharmacopoeia 8.0 (2014): 2.6.12. Microbial examination of non-sterile products (total viable aerobic count); 2.6.13 (B). Test for specified microorganisms.

EU GMP Medicinal Products for Human and Veterinary use (2008): Annex1 Manufacture of Sterile Medicinal Products.

Guidance for Industry (2004): Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice.

ISO 6579 (2002): Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

Official Journal of the European Union L338/1-26 (2005): Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs.

Taylor, W.I. (1965): Isolation of Shigellae. I. Xylose-Lysine Agar. New media for the isolation of enteric pathogens. *Am. J. Clin. Pathol.* **44**: 471-475.

United States Pharmacopoeia 38 NF 33 (2015): <61> Microbial Limit Tests; <62> Microbiological examination of non-sterile products: Tests for specified microorganisms.



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## Ordering Information

Product	Cat. No.	Pack size	Other pack sizes available
Xylose Lysine Deoxycholate (XLD) Agar – LI	1.46073.0020	20 x 90 mm	120 x 90 mm
Tryptic Soy Broth	1.46334.0006	6 x 90 ml	
Rappaport Vassiliadis Medium	1.46181.0020	20 x 10 ml	100 x 10 ml
Buffered Peptone Water	1.46403.0006	6 x 1000 ml	
Tetrathionate Broth (MKTn)	1.46221.0020	20 x 10 ml	100 x 10 ml
Rambach Agar (Salmonella-elective)	1.46719.0020	20 x 90 mm	100 x 90 mm
Brilliant Green, Phenol Red, Lactose, Sucrose (BPLS) Agar – LI	1.46046.0020	20 x 90 mm	
Deoxycholate Citrate Agar acc. to LEIFSON, mod.	1.02896.0500	500 g	
Hektoen Enteric Agar	1.11681.0500	500 g	
ReadyTube™ 12 Modified Semi-Solid Rappaport-Vassiliadis (MSRV) Medium acc. ISO 6579	1.46622.0100	100 x 12 ml	
Salmonella Shigella (SS) Agar	1.07667.0500	500 g	
Triple Sugar Iron Agar	1.03915.0500	500 g	
Tryptophan Broth	1.46731.0020	20 x 10 ml	

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