Technical Data Sheet

HEIMPLATE™ XLD Agar EP+USP

Ordering number: 1.46073.0020

Is a selective culture medium for isolation of Salmonella and Shigella in pharmaceuticals and clinical materials.

General

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 (g/l)

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.

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Application and Interpretation

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 for 18-24 hours at 30-35 °C. From this culture 0.1 ml a re transferred into 10 ml Rappaport Vassiliadis Broth and incubated for 18-24 hours at 30-35 °C. At least a subculture from Rappapor t 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.

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

Colony Characteristics	Indicator Reactions	Xyl	Lys	Lac / Suc	H2S	Possible Microorganisms
red, translucent colonies	no color change, no formation of iron sulfide	-	-	-	-	*Shigella, Providencia Pseudomonas spp.*
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	+	+	-	+/-	*Salmonella Edwardsiella spp.*
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	+	+	-	-	*Salmonella* Parathyphi A, H2S-negative *Salmonella*
yellow, opaque colonies on yellow medium (weak black center *Citrobacter* and possibly at *Proteus*)	acidification, color change to yellow, no formation of iron sulfide (possible is a slight formation of iron sulfide for *Citrobacter* and *Proteus*)	+/-	+/-	+	+/-	*E. coli Enterobacter Klebsiella Serratia Citrobacter Proteus spp*.

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 as well as further selective media like BPLS Agar, LEIFSON Agar (article number 102896), Hektoen Enteric Agar (article number 111681), Modified Semi-Solid Rappaport-Vassiliadis

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(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
Salmonella 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
Salmonella 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
Escherichia coli	8739	10-100	18-24 h at 35-37 °C	Recovery ≤ 5 %
Staphylococcus aureus	6538	10,000-100,000	18-24 h at 35-37 °C	No growth
Enterococcus faecalis	19433	10,000-100,000	18-24 h at 35-37 °C	No growth

Please refer to the actual batch related Certificate of Analysis.

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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.

Ordering Information

Product	Cat. No.	Pack size
HEIMPLATE™ XLD Agar EP+USP	1.46073.0020	20 x 90 mm

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