XLD AGAR (XYLOSE LYSINE DEOXYCHOLATE) ISO

AGXL-00I-500

- **Principle**

XLD Agar (Xylose Lysine Deoxycholate Agar) is prepared according to the formulation of the ISO 6579 norm. It is recommended for the identification of Salmonella in food products, after pre-enrichment in a non-selective fluid such as Buffered Peptone Water (AGPT-0DI-500) and enrichment in a selective fluid medium such as Muller Kauffmann Broth Base with Brilliant Green and Novobiocin (MKTTN) (MFKB-00I-500), Rappaport Soy Broth (Vassiliadis) (RSVB-00I-500).

The reactions are the degradation of the three fermentable carbohydrates: xylose, lactose, and sucrose, with the production of acid, manifested in the colour change from red to yellow. Sodium thiosulfate serves as a reactive substance with Ferric ammonium citrate as an indicator of the formation of hydrogen sulphide under alkaline conditions. Lysine is included to enable the Salmonella group to be differentiated from the non-pathogens since, in its absence, salmonellae would quickly ferment the xylose, making it indistinguishable from non-pathogenic species. After the salmonellae terminate the xylose present, the lysine is attacked through the enzyme lysine decarboxylase with a change to an alkaline pH, similar to the Shigella reaction. The bacteria that decarboxylate the L-Lysine to cadaverine are identified by the presence of a purple-red colour around the colonies due to the elevation of the pH. Phenol red is the pH indicator. Yeast extract is a source of vitamins, particularly of the B-group essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium deoxycholate is the selective agent and is thus inhibitory to Gram-positive microorganisms. Bacteriological Agar is the solidifying agent.

Typical colonies of Salmonella on XLD agar have a black centre and lightly transparent zone of reddish colour due to the colour change of the indicator. Salmonella H_2S-negative variants grown on XLD agar are pink with a darker pink centre. Lactose-positive Salmonella grown on XLD agar are yellow with or without blackening.

- **Regulatory compliance**

This product is manufactured under a quality management system in accordance with ISO 9001 and ISO 13485, and its formulation and quality control comply with applicable international standards, such as ISO 11133, where relevant.

For this specific medium, compliance is also established with the relevant requirements of ISO 19250, ISO 21557 and ISO 6579.

- **Composition**

Ingredients	**g/L**
Bacteriological agar	13.50
Lactose	7.50
Phenol red	0.08
Sodium deoxycholate	1.00
Sucrose	7.50
Yeast extract	3.00

Ferric ammonium citrate	0.80
L-Lysine hydrochloride	5.00
Sodium chloride	5.00
Sodium thiosulfate	6.80
Xylose	3.75

- **Preparation**

Suspend 54 grams of the medium in one litre of distilled water Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Cool the medium according to the applicable normative and pour into Petri dishes as soon as it has cooled.

Preparation of large volumes, overheating and prolonged storage in water bath is to be avoided. Precipitates may be formed but do not affect the performance of the culture media.

- **Applications and use**

For detection of Salmonella spp. in food, animal feed, animal faeces, and environmental samples according to ISO 6579:

- Preenrichment in non-selective liquid medium: Inoculate the Buffered Peptone Water (AGPT-0DI-500) with the sample or dilutions and incubate at 34-38 °C for 18±2 h.

- Enrichment in/on selective media: Inoculate, with the culture obtained in the pre-enrichment stage, the Rappaport Soy Broth (Vassiliadis) (RSVB-00I-500) or the MKKTN Broth (MKFB-00I-500). The Rappaport Soy Broth and the Modified Semisolid Rappaport medium are incubated at 41.5 °C for 24±3 h, and the MKKTN Broth at 34-38 °C for 24±3 h.

- Plating out on selective solid media: From the selective enriched cultures, inoculate two selective isolation agars; XLD agar (AGXL-00I-500) and any other selective medium complementary to XLD agar (Brilliant Green Agar (BGAG-00I-500), Hektoen Enteric Agar (HEAG-00P-500), Salmonella Shigella Agar (SSAG-IEP-500). Incubate the XLD plates inverted at 34-38 °C for 24±3 h. Incubate the second selective medium in accordance with the manufacturer's instructions.

- Confirmation: Subculture colonies of presumptive Salmonella and confirm their identity by biochemicals and serological tests.

Note: According to Annex D of ISO 6579-1: 2017, for the detection of enterica subspecies enterica serovars Typhi and Paratiphy, Selenite Cystine Broth should be added as a selective enrichment medium, and Bismuth Sulphite Agar (Wilson Blair) should be selected as a second selective medium.

For detection of Salmonella spp. in water samples according to ISO 19250:

- Preenrichment in non-selective medium: Inoculate the Buffered Peptone Water (AGPT-0DI-500) with the sample or dilutions and incubate at 34-38 °C for 18±2 h.

- Enrichment in selective media: Inoculate, with the culture obtained in the pre-enrichment stage, the Rappaport Soy Broth (Vassiliadis) (RSVB-00I-500) and the MKKTN Broth (MKFB-00I-500). The Rappaport Soy Broth is incubated at 41.5±1 °C and the MKKTN Broth at 34-38 °C, both for 24±3 hours.

- Plating out on selective solid media: From the selective enriched cultures, inoculate two selective isolation agars; XLD agar (AGXL-00I-500) and any other selective medium complementary to XLD agar (For instance, Brilliant Green Agar (BGAG-00I-500)) Incubate the XLD plates inverted at 34-38 °C for 24±3 hours. Incubate the second selective medium in accordance with the manufacturer's instructions.

- Confirmation: Subculture colonies of presumptive Salmonella and confirm their identity by biochemicals and serological tests.

- **Quality control**

Solubility	w/o rests
Appearance	Fine powder
Colour of the dehydrated medium	Pink
Colour of the prepared medium	Red-orange
Final pH (25 °C)	7.4 ± 0.2

- **Microbiological test**

According to ISO 11133:

Incubation conditions: Productivity, Selectivity ISO 6579 (34-38 °C / 24±3 h). Productivity, Selectivity ISO 19250 (36±2 °C / 24±3 h).

Inoculation conditions: Productivity (10^3-10^4 CFU), Selectivity (10^4-10^6 CFU).

Microorganisms	ATCC	Specification	Characteristic reaction
Salmonella enteritidis	13076	Good growth (2)	Colonies with black centre and a lightly transparent zone of reddish colour due to the colour change of the medium
Salmonella typhimurium	14028	Good growth (2)	Colonies with black centre and a lightly transparent zone of reddish colour due to the colour change of the medium
Escherichia coli	25922	Growth or partial inhibition (0-1)	Yellow colonies
Enterococcus faecalis	29212	Total inhibition (0)	-

- **Storage**

The product is highly hygroscopic; keep the container always closed and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Temp. Min.:2 °C Temp. Max.:25 °C.

Note: Sterilize media immediately after reconstitution.

- **Bibliography**

International Standard UNE-EN-ISO 6579. Food Microbiology for human consumption and Animal Feed. Horizontal Method for the detection of *Salmonella* spp.

ISO 19250 water quality-detection of *Salmonella* spp

ISO 6579 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC.

- **Product use limitation**

This product is developed, designed and supplied exclusively for research use only. It is not intended for diagnostic applications or drug development, and it is not suitable for administration to humans or animals.