XLD AGAR
ACCORDING TO ISO 6579-1:2017

INSTRUCTION FOR USE

For professional use

Intended use: XLD Agar is used for the isolation and differentiation of Salmonella spp. from food, animal feed samples and environmental samples from food.

Ref.	Type of medium:	Packaging:
8013DM	dehydrated medium	500 g
8013BT	ready-to-use medium-bottle	100, 200, 500 ml
8013PD	ready-to-use medium-plate	1x10 pcs (90 mm)

1. Principle: yeast extract provides sources of nitrogen, carbon, and vitamins required for organism growth. Xylose, lactose, and sucrose provide sources of fermentable carbohydrate. Xylose is fermented by most enteric organisms except *Shigella* spp. and *Providencia* spp. L-lysine hydrochloride is added to differentiate *Salmonella*. As xylose is exhausted, *Salmonella* spp. organisms decarboxylate lysine causing a reversion to alkaline conditions. Alkaline reversion by other lysine-positive organisms is prevented by excess acid production from fermentation of lactose and sucrose. Sodium thiosulfate and iron (III) ammonium citrate act as selective agents, allowing visualization of hydrogen sulfide production under alkaline conditions. Sodium deoxycholate is also a selective agent. Phenol red is an indicator. Sodium chloride maintains the osmotic balance in the medium. Agar is the solidifying agent.

2. Formula/Liter:

Yeast extract	3.0 g
Sodium chloride	5.0 g
Xylose	3.75 g
Lactose	7.5 g
Sucrose	7.5 g
L-lysine hydrochloride	5.0 g
Sodium thiosulfate	6.8 g
Iron (III) ammonium citrate	0.8 g
Phenol red	0.08 g
Sodium deoxycholate	1.0 g
Agar	13.0 g

3. pH: 7.4 ± 0.2 at 25ºC.

4. Preparation:

Dehydrated medium:
Suspend 55.0 g of the medium in one liter of purified water. Heat to boiling with frequent agitation until completely dissolved. Do not autoclave, do not overheat. Some turbidity may occur after boiling it normally disappears when the temperature decreases to 45-50°C and doesn't affect the medium performance. Pour into sterile Petri dishes.*

Bottles with agar:
Agar in the bottles should be melted in water batch at 80ºC or microwaved.

Melting of agar base in microwave
1. Loosen the cap on the agar bottle before microwaving.
2. Place the bottle with agar base in central place of microwave.
3. Heat in one-minute intervals on low power until all of the agar is melted.
4. Between intervals, gently swirl the bottle to make sure the agar is melting evenly.
5. While wearing heat-protective gloves, carefully remove the hot bottle and let it cool to 45-50°C. Some turbidity may occur after boiling; it normally disappears after cooling and doesn't affect the medium performance.
6. Pour on Petri dishes.*

Melting of agar base in water bath

1. Loosen the cap on the agar bottle and place it into the water bath.
2. Keep the water temperature at around 80°C.
3. Leave the bottle with the agar base in the water bath until the agar is completely melted.
4. While wearing heat-protective gloves, carefully remove the hot bottle, stir it gently and let it cool to 45-50°C. Some turbidity may occur after boiling, it normally disappears after cooling and doesn't affect the medium performance.
5. Pour on Petri dishes.*

** Working in a clean, draft-free area disinfected with bactericidal cleaner. Take care to use aseptic techniques to prevent contamination. Working on one plate at a time, carefully tilt open the cover and pour about 15–20 ml of liquid into the bottom portion (it should cover about 2/3 of the plate's surface). Gently rotate the dish to ensure that the liquid medium covers the base of the dish evenly. The layer should be about 3–4 mm deep. Allow plates to solidify and cool before use. This takes about one hour. Do not put agar plates in a freezer to speed up this process. Dry the plates with the lid slightly off for 20 minutes in the laminar flow hood or a 37°C incubator to avoid water evaporation and condensation on the lid during storage or incubation.*

5. **Appearance:**

Dehydrated Appearance: dehydrated medium is homogeneous, free flowing, and pink-beige. Some turbidity may occur after boiling; it normally disappears after cooling and doesn't affect the medium performance.

Prepared Appearance: prepared medium is clear and light orange-red. Some turbidity may occur after boiling, it normally disappears after cooling and doesn't affect the medium performance.

6. Sample: all samples in which a enteric pathogens are expected.

7. Test procedure: if the agar plate has been refrigerated, allow to warm to room temperature before inoculation. From the culture obtained in the RSV Broth (Ref:. 6011), inoculate by means of 10 µl loop the surface of a XLD Agar, so that well-isolated colonies will be obtained. From the positive growth obtained on the MSRV Agar, determine the furthest point of opaque growth from inoculation points and dip a 1 µl loop just inside the border of the opaque growth. Withdraw the loop ensuring that no large lumps of MSRV Agar (Ref:. 3085) are extracted. Inoculate the surface of a XLD Agar , so that well-isolated colonies will be obtained. From the culture obtained in the MKKTm Broth (Ref:. 6092) inoculate by means of 10 µl loop the surface of a XLD Agar, so that well-isolated colonies will be obtained. Incubate the XLD agar at 37°C for 24±3h.

8. Results: after incubation time observe growth of characteristic microorganisms. Identification of the microorganism should be confirmed by biochemical test.

9. Quality control: perform quality control testing for both negative and positive reaction by inoculating a representative sample of plates with pure cultures of stable control organisms that produce known, desired reactions according to ISO 11133:2014.

Microorganism:	**Method of control:**	**Recovery:**	**Reaction:**
Salmonella typhimurium WDCM 00031	recovery: qualitative	good growth (2)	colonies with black centre and a lightly transparent zone of reddish colour change of medium.
Salmonella enteritidis WDCM 00030	recovery: qualitative	good growth (2)	colonies with black centre and a lightly transparent zone of reddish colour change of medium.
Escherichia coli WDCM 00013	selectivity: qualitative	growth or partial inhibition (0-1)	yellow colonies
Enterococcus faecalis WDCM 00087	selectivity: qualitative	toatl inhibition (0)	___

10. Precautions: due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium

11. Disposal of waste: after use, all plates and any other contaminated materials must be sterilized or disposed of in line with appropriate internal procedures and in accordance with local legislations. Plates can be destroyed by autoclaving at 121°C for at least 20 minutes.

12. Storage: sealed, unopened containers with dehydrated powdered media should be stored at 2-30°C. Once opened and recapped, place the container in a low humidity environment at room temperature. Protect from moisture and light.
Bottles should be stored at 6-25°C in the dark used before the expiry date on the label. On receipt, store plates at 2-12°C away from direct sun light in an inverted position. Do not overload a refrigerator with excessive amounts of plates to avoid water condensation on the lids during storage. Plates must not come into direct contact with the inner walls of refrigerator, as the media may freeze, invalidating the tests. Prepared plates, stored in their original sleeve wrapping at 2-12°C until just prior to use, may be inoculated up to the expiration date and incubated for recommended incubation times. Plates from opened stacks of 10 plates should be used for two weeks when stored in a clean area at 2 to 12° C.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or others signs of deterioration. Allow the medium to warm to the room temperature before inoculation.
All microbiological media containing dyes or light-sensitive components should be protected from light and stored in the dark.

Note that shelf life of the growth media changes after the addition of supplements. Complete media containing protein supplement tend to degrade faster than basal media alone.

13. Shelf life: dehydrated medium: 3 years,
bottles: 1 year,
plates: 3 months.

14. Required supplements not supplied together with medium base: not applicable.

15. References: available on request.