

## UPUTSTVO ZA UPOTREBU

(SRB)

### XLD - modifikovani Agar Plate

Selektivna podloga za izolaciju i enumeraciju *Salmonella Typhi* i drugih *Salmonella* vrsta.

#### Sadržaj pakovanja:

| Šifra artikla (pakovanja) | Opis                                     | Šifra primarnog pakovanja: | Broj podloga |
|---------------------------|--|----------------------------|--------------|
| PRM031IV20                | Podloga izlivena u petri posudama od ø90 | PRM031I                    | 20           |
| PRM031IV60                |  |                            | 60           |
| PRM031IV240               |  |                            | 240          |
| PRM031IM40                | Podloga izlivena u petri posudama od ø50 |                            | 40           |

#### Uputstva

Pod aseptičnim uslovima inokulisati ploču metodom površinskog zasejavanja. Nakon inkubacije posmatrati rast i boju kolonija.

#### Princip i interpretacija

Xylose Lysine Deoxycholate (XLD) agar je napravio Taylor (1-5) za izolaciju i diferencijaciju enteričnih patogena uključujući *Salmonella Typhi* od drugih vrsta roda *Salmonella*. XLD Agar, modifikovan se preporučuje za selektivnu izolaciju i enumeraciju *Salmonella Typhi* i drugih *Salmonella* vrsta u skladu sa ISO Committee (20).

XLD agar je podloga pojačane selektivnosti i osetljivosti u poređenju sa drugim podlogama kao što su: SS Agar, EMB Agar i Bizmut-sulfitni agar (2,4,6 i 13-16). Formulacija podloge onemogućava prerastenje *Salmonella* spp. i *Shigella* spp. od strane drugih mikroorganizama (17).

Podloga sadrži ekstrakt kvasca koji obezbeđuje azot i vitamine potrebne za rast bakterija. Ksilozu, lakozu i saharozu su izvor potencijalno fermentabilnih ugljenih hidrata, međutim ksilozu inkorporiranu u podlogu *Shigella* vrste ne fermentiše, za razliku od drugih enterobakterija. Ova osobina pomaže u diferencijaciji *Shigella* spp. Natrijum-hlorid održava osmotski balans podloge. Lizin je vezan za diferencijaciju salmonela od ostalih ne-patogena. *Salmonella* spp. brzo fermentiše ksilozu i iscrpljuju je kao izvor ugljenika. Zatim se lizin enzimom lizin dekarboksilazom dekarboksiliše dajući amine koji alkalnom reakcijom poništavaju promenu pH podloge oponašajući tako reakciju koju daje *Shigella* spp. Da bi se prevazišla lažna reakcija koju izazivaju lizin-pozitivni koliformi, podlozi su dodati saharozu i lakozu koji obezbeđuju dodatnu produkciju kiselina. Razgradnja ksiloze, lakoze i saharoze do kiselina uzrokuje promenu boje indikatora fenol-crvenog u žutu. Bakterije koje dekarboksilišu lizin u kadaverin mogu se prepoznati po crvenoj obojenosti oko kolonija, koja se javlja usled povećanje pH. Ove reakcije se mogu odvijati istovremeno ili sukcesivno, što može prouzrokovati različite nijanse boja indikatora ili promenu boje iz žute u crvenu pri produženoj inkubaciji.

Da bi se obezbedila diferencijacija kolonija u podlogu su kao H<sub>2</sub>S-indikatori uključeni natrijum tiosulfat i gvožđe ammonijum citrat koji obezbeđuju vizualizaciju produkcije H<sub>2</sub>S, a što se manifestuje formiranjem kolonija sa crnim centrom. Ne-patogene bakterije koje produkuju H<sub>2</sub>S ne dekarboksilišu lizin zbog čega produkcija kiselina u njihovom slučaju onemogućava crnjenje kolonija (1).

XLD agar je i diferencijalna i selektivna podloga. Natrijum deoksiholat je selektivni agens kao inhibitor Gram-pozitivnih organizama. Neki sojevi *Proteus* vrsta mogu da daju crveno do žuto obojenje sa kolonijama koje imaju crni centar, što doprinosi lažno pozitivnim rezultatima. Ne-enterični organizmi poput *Pseudomonas* spp. i *Providencia* spp. mogu dati crvene kolonije. S. Paratyphi A, S. Choleraesuis , S. Pullorum i S. Gallinarum mogu da formiraju crvene kolonije bez H<sub>2</sub>S koje liče na kolonije *Shigella* vrste (19).

#### Kontrola kvaliteta

Podaci i rezultati kontrole kvaliteta dati su u sertifikatu analize za svaku seriju.

#### Skladištenje i rok upotrebe

Čuvati između 15-25°C. Nakon prvog otvaranja čuvati na 2-8°C. Upotrebiti pre isteka datuma označenog na nalepnici

#### Mere predostrožnosti

Ovaj proizvod ne sadrži hazardne supstance u koncentracijama koje su iznad propisanih limita određenih važećim zakonskim regulativama i zato nije klasifikovan kao opasan. Ipak, preporučeno je slediti smernice iz bezbednosnog lista za pravilnu upotrebu. Ovaj proizvod je namenjen isključivo za upotrebu u laboratorijskim uslovima, od strane profesionalno obučene osobe.

Proizvod ne upotrebljavati ukoliko je primarno pakovanje oštećeno ili proizvod ne odgovara navedenim karakteristikama.

#### Odlaganje otpada

Odlaganje otpada mora biti u skladu sa nacionalnim i lokalnim regulativama koje su na snazi. Svaka laboratorija je odgovorna za rukovanje i odlaganje otpada koji nastaje u toku rada.

#### Upotrebljeni simboli

|  |                                       |  |                     |
|--|---------------------------------------|--|---------------------|
|  | Držati uspravno                       |  | Kataloški broj      |
|  | Ne izlagati direktno sunčevim zracima |  | Lot broj            |
|  | Konsultovati uputstvo za upotrebu     |  | Rok upotebe         |
|  | Ne koristiti više puta                |  | Temperatura čuvanja |
|  | Veličina pakovanja                    |  | Proizvođač          |

#### Literatura

1. Taylor W. L., 1965, Am. J. Clin. Pathol., 44:471-475.
2. Taylor W. L. and Harris B., 1965, Am. J. Clin. Pathol., 44:476.
3. Taylor W. L. and Harris B., 1967, Am. J. Clin. Pathol., 48:350.
4. Taylor W. L. and Schelhart B., 1967, Am. J. Clin. Pathol., 48:356.
5. Taylor W. L. and Schelhart B., 1968, Am. J. Clin. Pathol., 16:1387.
6. Taylor W. L. and Schelhart B., 1969, Appl. Microbiol., 18:393-395.
7. Chadwick P., Delisle G. H and Byer M., 1974, Can. J. Microbiol., 20, 1653-1664.
8. Downes F. P. and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological examination of Foods, 4th Ed., APHA Inc. Washington D.C.
9. Eaton A. D., Clesceri L. S., Rice E. W., and Greenberg A. W., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.
10. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
11. Williams H., (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C.
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13. Dunn C. and Martin W. J., 1971, Appl. Microbiol., 22, 17-22.
14. Rollender M. A., Beckford O., Belsky R. D and Kostroff B. 1969, Am. J. Clin. Pathol., 51, 284-286.
15. Taylor W. L. and Schelhart B., 1969, Appl. Micro. 18, 1387-1392.
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17. Isenberg H. D., Kominos S., and Sigeal M., 1969, Appl Microbiol., 18, 656-659.
18. Aspinall S. T., Hindle M. A. and Hutchinson D. N., 1992, 19. Eur. J. Clin. Microbiol., Inf. Dis. 11, 936-939. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
20. International Organization for Standardization (ISO), 2002, Draft ISO/DIS 6579:2002.

## INSTRUCTION FOR USE

(EN)

### XLD Agar- Modified Agar Plate

Media for selective isolation and enumeration of *Salmonella Typhi* and other *Salmonella* species.

#### Package contents:

| Item code<br>(packaging) REF | Description                               | Primary<br>packaging<br>code: | Number of<br>products |
|------------------------------|---|-------------------------------|-----------------------|
| PRM031IV20                   | Substrate poured into petri dishes of ø90 | PRM031                        | 20                    |
| PRM031IV60                   |   |                               | 60                    |
| PRM031IV240                  |   |                               | 240                   |
| PRM031IM40                   | Substrate poured into petri dishes of ø50 |                               | 40                    |

#### Directions

Surface spread the test inoculum aseptically on the plate. After incubation, observe growth and color of colonies.

#### Principle And Interpretation

XLD Agar was formulated by Taylor (4-8) for the isolation and differentiation of enteric pathogens including *Salmonella Typhi* from other *Salmonella* species. XLD Agar, Modified is recommended for selective isolation and enumeration of *Salmonella Typhi* from other *Salmonella* species in accordance with ISO Committee (3).

XLD agar has enhanced selectivity and sensitivity compared to other media such as: SS Agar, EMB Agar and Bismuth sulphite agar. Its formulation prevents the overgrowth of *Salmonella* spp. and *Shigella* spp. by other microorganisms (2).

This medium contains yeast extract, which provides nitrogen and vitamins required for growth. Xylose, lactose and sucrose are sources of fermentable carbohydrates, however xylose is mainly incorporated into the medium since it is not fermented by *Shigella* spp. but practically by all enterobacteria. This helps in the differentiation of *Shigella* species. Sodium chloride maintains the osmotic balance of the medium. Lysine is included to differentiate the *Salmonella* group from the non-pathogens. *Salmonella* spp rapidly ferment xylose and exhaust carbon source. Subsequently lysine is decarboxylated by the enzyme lysine decarboxylase to form amines with reversion to an alkaline pH that mimics the *Shigella* spp. reaction. However, to prevent this reaction by lysine-positive coliforms, lactose and sucrose are added to produce acid in excess. Degradation of xylose, lactose and sucrose to acid causes phenol red indicator to change its colour to yellow. Bacteria that decarboxylate lysine to cadaverine can be recognized by the appearance of a red colouration around the colonies due to an increase in pH. These reactions can proceed simultaneously or successively, and this may cause the pH indicator to exhibit various shades of colour or it may change its colour from yellow to red on prolonged incubation.

In order to ensure the differentiation of colonies, sodium thiosulphate and ferric ammonium citrate are included in the medium as H<sub>2</sub>S-indicators, which provide visualization of H<sub>2</sub>S production, which is manifested by the formation of colonies with a black center. Non-pathogenic bacteria that produce H<sub>2</sub>S do not decarboxylate lysine, which is why the production of acids in their case prevents blackening of colonies (1).

XLD agar is both a differential and a selective medium. Sodium deoxycholate is a selective agent as an inhibitor of Gram-positive microorganisms.

#### Quality control

The data and results of quality control are given in the certificate of analysis for each lot.

#### Storage and shelf life

Storage between 15-25°C. After opening store between 2-8°C . Use before expiry date on the label.

#### Warning and precautions

This product does not contain hazardous substances in concentrations that are above the prescribed limits set by applicable legislation and are therefore not classified as hazardous. However, it is recommended to follow the guidelines provided in the safety data sheet for proper use. This product is intended for laboratory use only by a professionally trained person.

Do not use the product if the primary packaging is damaged or the product does not meet the stated characteristics.

#### Disposal

Waste disposal must be in accordance with national and local regulations. Each laboratory is responsible for handling and disposing of waste generated during operation.

#### Symbols used on labels

|  |                                    |  |                   |
|--|------------------------------------|--|-------------------|
|  | This side up                       |  | Catalogue number  |
|  | Do not expose directly to sunlight |  | Batch code        |
|  | Consult instructions for use       |  | Use-by date       |
|  | Do not re-use                      |  | Temperature limit |
|  | Pack size                          |  | Manufacturer      |

#### Reference

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