HEKTOEN ENTERIC AGAR

- For in vitro use only - Catalogue No. PH45

Our Hektoen Enteric Agar is used for the isolation and differentiation of gram-negative enteric bacilli, particularly *Salmonella* and *Shigella* species.

King and Metzger developed Hektoen Enteric Agar in 1967, and found that their medium was conducive for growth of *Salmonella* and *Shigella* species while inhibiting normal intestinal flora. Our current formulation adheres to APHA specifications and differs slightly from the original formulation. The elimination of desoxycholate and reduction in the bile salt concentration results in better isolation of enteric bacilli while maintaining the medium's high selectivity.

The nutritional components of Hektoen Agar include a mixture of meat peptone, yeast extract, along with the carbohydrates lactose, sucrose, and salicin. The relatively high levels of carbohydrates and peptone counteract the inhibitory effects that bile salts may have on certain organisms, such as *Shigella* species. The inclusion of carbohydrates along with the pH indicators, bromthymol blue and acid fuchsin, allow for differentiation of organisms based on their sugar fermentation patterns; Fermentation of any or all three types of carbohydrates present will result in acid production and a change in the pH indicator with a corresponding change in the color of the medium. Lactose-sucrose-salicin fermenters produce bright yellow or orange colored colonies, while non-fermenters produce blue-green colored colonies.

Formula per Litre of Medium

Meat peptone	12.0 g
Yeast extract	3.0 g
Bile salts No. 3	9.0 g
Lactose	12.0 g
Sucrose	12.0 g
Salicin	2.0 g
Sodium chloride	5.0 g
Sodium thiosulfate	5.0 g
Ferric ammonium citrate	1.5 g
Agar	14.0 g
Acid fuchsin	0.1 g
Bromothymol blue	0.065 g

pH 7.5 ± 0.2

Recommended Procedure

1. Allow medium to reach room temperature prior to inoculation.
2. Using an inoculum from the specimen, perform a four-quadrant streak to obtain well-isolated colonies.
3. Incubate aerobically at 35°C.
4. Examine after 18-24 hours. Incubate plates an additional 24 hours if required.

Interpretation of Results

Shigella and *Salmonella* species do not ferment lactose, sucrose, or salicin and therefore colonies appear green to blue-green in color. S*almonella* species that are H_2S positive will also have black centers.

Most coliforms are inhibited on Hektoen Enteric Agar, but species may overcome the inhibitory effects of the medium after prolonged incubation. Coliforms that can ferment the available carbohydrates, such as *Escherichia coli*, will produce orange to salmon-coloured colonies.

Additional biochemical and/or serological tests should be performed on isolated colonies from pure culture in order to complete identification.

- *Proteus species that prove capable of growing on this medium will form colonies resembling Shigella colonies*

- *Bile salts may crystallize over time, and appear as white spider-like deposits in the medium. This does not affect the performance of the medium*

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Result
Salmonella typhimurium ATCC 14028	Growth, green to blue-green colonies with or without black centers
Shigella flexneri ATCC 12022	Growth, green to blue-green colonies
Escherichia coli ATCC 25922	Partial inhibition, orange to salmon-colored colonies
Enterococcus faecalis ATCC 29212	Marked or complete inhibition

Storage and Shelf Life

Our Hektoen Enteric Agar should be stored away from direct light at 4 to 8°C with the medium side uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this medium has a shelf life of 7 weeks from the date of manufacture.

References

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