Technical Data Sheet

CE Hektoen Enteric Agar

Ordering number: 1.11681.0500

Hektoen Enteric Agar is a selective agar proposed by King and Metzger (1968) for detecting and isolating pathogenic intestinal bacteria including Salmonella and Shigella in various materials such as faeces, foodstuffs etc.

When compared with other selective culture media (e.g. SS Agar, BPL Agar and Bismuth Sulfite Agar), Hektoen Enteric agar has the advantage that it only slightly inhibits the growth of *Salmonella* and *Shigella* thus giving high yields of these microorganisms, but at the same time ensures adequate inhibition of accompanying microorganisms (King and Metzger 1968, Taylor and Schelhart 1971, Bisciello and Schrade 1974).

Hektoen Enteric Agar is a medium of moderate to high selectivity and it can be used routinely in combination with a low selective medium for the optimal isolation of Shigella. To enhance the recovery of species of Salmonella and Shigella from heavy numbers of mixed normal fecal flora, Hektoen Enteric Agar is used as both a direct and indirect plating medium for fecal specimens.

IVD in vitro diagnosticum - For professional use only

Mode of Action

Lactose-positive colonies have a clearly different color from lactose-negative colonies due to the presence of the two indicators bromothymol blue and acidic fuchsin. This color difference is also observed for colonies, which can only slowly ferment lactose due to the presence of sucrose and salicin. These reactive compounds can be fermented more easily - false-positive pathogenic results are thus avoided. The combination of thiosulfate as a reactive compound with an iron salt as an indicator causes H_2S-positive colonies to become black in color. The mixture of bile salts suppresses the growth of most of the accompanying microorganisms.

Hoben et al. (1973) recommended addition of 10-20 µg novobiocin/ml to the medium to improve its selectivity i.e. to inhibit *Citrobacter* and *Proteus* colonies which resemble those of *Salmonella* (black center).

Typical Composition

Peptones	15 g/l
NaCl	5 g/l
Yeast Extract	3 g/l
Sucrose	14 g/l
Lactose	14 g/l

Salicin	2 g/l
Sodium Thisulfate	5 g/l
Ammonium Iron(III) Citrate	1.5 g/l
Bile Salt Mixture	2.0 g/l
Bromothymol Blue	0.05 g/l
Acidic Fuchsin	0.08 g/l
Agar-Agar	13.5 g/l

Preparation

Suspend 75 g in 1 l of demineralized water and let soak for 10 min. Gently heat and bring to boil for a few seconds to dissolve the medium completely. **Do not autoclave**.

If desired, add 15 mg novobiocin/l to the cooled (50 °C) medium in form of a filter-sterilized solution. Pour plates.

The appearance of the prepared plates is clear and blue-green

The pH at 25 °C is in the range of 7.5-7.9.

Specimen

e.g. Stool.

Clinical specimen collection, handling and processing. See general instructions of use.

Experimental Procedure and Evaluation

Inoculate the culture medium with material taken from an enrichment culture by spreading thinly on the surface of the plates.

Incubation: 18-24 h at 35-37 °C aerobically.

Colonies of the most important bacteria usually have the appearance described below. Colonies which are suspected to be pathogenic should be subjected to further tests to confirm their identity.

Appearance of Colonies	**Microorganisms**
Green, moist, flat, transparent	*Shigella, Providencia*
Blue-green, with or without a black centre	*Salmonella, Paracolobactum, Proteus*
Green to bluish, flat, irregular edge	*Pseudomonas*
Orange-red surrounded by a zone of precipitate	*Coliform* bacteria

Storage

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.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

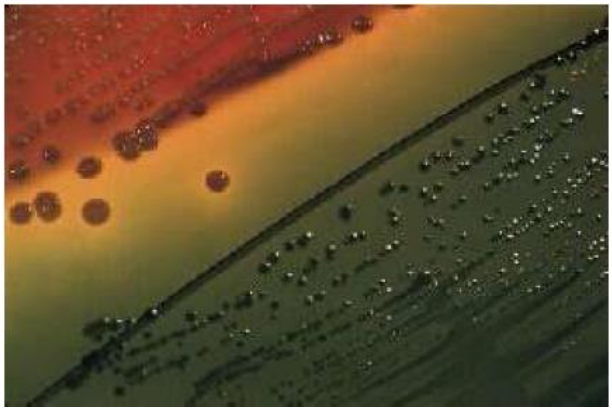
Disposal

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 #	Incubation	Expected Results
Salmonella typhimurium	14028	18-24 h at 35-37 °C	Growth, blue-green colony color, black center, no precipitation
Salmonella enteritidis	13076	18-24 h at 35-37 °C	Growth, blue-green colony color, black center, no precipitation
Shigella flexneri	12022	18-24 h at 35-37 °C	Growth, green to blue-green colony color, no black center, no precipitation
Shigella sonnei	11060	18-24 h at 35-37 °C	Growth, green to blue-green colony color, no black center, no precipitation
Proteus mirabilis	14273	18-24 h at 35-37 °C	Growth, green to blue-green colony color, black center +/-, no precipitation
Escherichia coli	25922	18-24 h at 35-37 °C	Growth, orange red colony color, no black center, precipitation
Staphylococcus aureus	25923	18-24 h at 35-37 °C	No growth
Enterococcus faecalis	29212	18-24 h at 35-37 °C	Growth inhibited

Please refer to the actual batch related Certificate of Analysis.



Shigella flexneri, Escherichia coli

Literature

Bisciello, N.B. jr. and Schrade, J. (1974). Evaluation of Hektoen Enteric Agar for the detection of *Salmonella* in foods and feeds. Journ. of AOAC, **57**: 992-996.

Hoben, D.A., Ashton, D.H. and Petersen, A.C. (1973). Some observations on the incorporation of novobiocin into Hektoen Enteric Agar for improved *Salmonella* isolation. Appl. Microbiol. **26**: 126-127.

King, S. and Metzger, W.J. (1968): A new plating medium for the isolation of enteric pathogens. I. Hektoen Enteric Agar. Appl. Mikrobiol. **16**: 557-578.

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Taylor, W.I. and Schelhart, D. (1971). Isolation of *Shigellae*, VII. Comparison of Xylose Lysine Deoxycholate Agar, Hektoen Enteric Agar, *Salmonella-Shigella* Agar and Eosin Methylene Blue Agar with stool specimen. Appl. Microbiol. **21**: 32-37.

Ordering Information

Product	Cat. No.	Pack size
Hektoen Enteric Agar	1.11681.0500	500 g
Novobiocin, Sodium Salt - CAS 1476-53-5 - Calbiochem	491207-10GM	10 gm

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