

51490 Hektoen Enteric Agar

Selective agar for the detection and isolation of pathogenic intestinal bacteria including *Shigella* and *Salmonella*, from various sources such as food, stool etc.

Composition:

Ingredients	Grams/Litre
Mixed peptone	12.0
Yeast extract	3.0
Sucrose	12.0
Lactose	12.0
Salicin	2.0
Bile salts	9.0
Sodium chloride	5.0
Sodium thiosulfate	5.0
Ammonium ferric citrate	1.5
Acid fuchsin	0.1
Bromothymol blue	0.065
Agar	15.0
Final pH 7.5 +/- 0.2 (at 25°C)	

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Appearance: Faintly greenish-beige coloured, homogeneous, free flowing powder.

Gelling: Firm

Color and Clarity: Green coloured clear to slightly opalescent gel forms in petri plates.

Directions:

Suspend 76.67 g in 1 litre distilled water and let it soak. Heat up by constant stirring until boiling. Cool to 55 - 60°C and pour into sterile plates. Do not autoclave. The Medium is very thermolabile and thus overheating should be avoided.

Principle and Interpretation:

Hektoen Enteric Agar was originally developed by King and Metzger [1, 2]. It improved the detection of *Salmonella* and *Shigella* organisms compared to other enteric differentiating media. The high content of carbohydrate and peptone in the medium neutralise the inhibitory effects of the bile salts and indicators but the adequate inhibition of gram-positive microorganisms was still ensured. *Salmonella* contamination is the second leading cause of food-borne illness worldwide. Most outbreaks of *Salmonella* are traced back to dairy, poultry and meat products, but *Salmonella* can grow on nearly any food. Chicken, eggs and their derivative products are particularly high risk.

Mixed peptone and yeast extract are rich sources for nitrogenous compounds, vitamins, carbon, sulphur and amino acids. The carbohydrates sucrose, lactose and salicin are the fermentable substrates while bile salts, acid fuchsin and bromothymol blue inhibits gram-positive organisms. Sodium chloride is for the osmotic balance. Ammonium ferric citrate is a source of iron, which is used as indicator for the production of hydrogen sulfide (H₂S). Sodium thiosulfate is used as the substrate for building hydrogen sulphide which gives together with iron a black precipitate (Iron(II) sulphide; FeS). H₂S-positive colonies have black centers. The indicator bromothymol blue changes its colour to yellow and acid fuchsin would change color from yellow to pink when acid is formed. *Proteus* species may resemble *salmonellae* or *shigellae*. Further testing must be carried out to confirm the presumptive identification of organisms isolated on this medium.



Cultural characteristics after 18-24 hours at 35°C.

Organisms (ATCC)	Growth	H ₂ S production	Appearance of Colony
<i>Enterococcus faecalis</i> (29212)	-	-	-
<i>Escherichia coli</i> (11775)	-/+	-	salmon-orange, may with bile ppt.
<i>Salmonella typhimurium</i> (14028)	+++	+	greenish blue with black centers
<i>Shigella flexneri</i> (12022)	+++	-	greenish blue

References:

1. S. King, W.I. Metzger, A new plating medium for the isolation of enteric pathogens. Appl. Microbiol., 16, 577-578 (1968)
2. S. King, W.I. Metzger, A new plating medium for the isolation of enteric pathogens. II. Comparison of Hektoen Enteric Agar with SS and EMB Agar, Appl. Microbiol. 16, 579-581(1968)
3. W.I. Taylor, D. Schelhaut, Comparison of Xylose Lysine Desoxycholate Agar, Hektoen Enteric Agar, Salmonella-Shigella Agar, and Eosin Methylene Blue Agar with stool specimens, Appl. Microbiol., 21, 32-37 (1971)
4. D.A. Hoben, D.H. Ashton, A.C. Peterson, Some observations on the incorporation of novobiocin into Hektoen Enteric Agar for improved Salmonella isolation. Appl. Microbiol. 26, 126-127 (1973)
5. R.S. Flowers, J-Y. D'Aoust, W.H. Andrews, J.S. Bailey, C. Vanderzant, D.F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed., *Salmonella*, p. 371-422, American Public Health Association, Washington, D.C. (1992)
6. R.S. Flowers, W. Andrews, C.W. Donnelly, E. Koenig, Pathogens in milk and milk products, R.T. Marshall, (ed.), Standard methods for the examination of dairy products. 16th ed., p. 103-212, American Public Health Association, Washington, D.C. (1993)
7. L.D. Gray, *Escherichia*, *Salmonella*, *Shigella*, and *Yersinia*, P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, R.H. Tenover (ed.), Manual of clinical microbiology, 6th ed., p. 450-456, American Society for Microbiology, Washington, D.C. (1995)
8. W.H. Andrews, G.A. June, P.S. Sherrod, T.S. Hammack, R.M. Amaguana. *Salmonella*, Bacteriological analytical manual, 8th ed., p. 5.01-5.20, AOAC International, Gaithersburg, MD (1995)
9. Association of Official Analytical Chemists, official methods of analysis of AOAC International, AOAC International, Arlington, VA (1996)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

The vibrant M, Millipore, and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. Detailed information on trademarks is available via publicly accessible resources.
© 2018 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the US and Canada.

