

TM 121 -HEKTOEN ENTERIC AGAR (ISO 21567:2004)

INTENDED USE

For differential & selective isolation of *Salmonella* and *Shigella* from enteric pathological specimens.

PRODUCT SUMMARY AND EXPLANATION

Hektoen Enteric Agar was developed in 1967 by King and Metzger of the Hektoen Institute in order to increase the frequencies of isolation of *Shigella* and *Salmonella* organisms when compared with their recovery on other media frequently utilized in clinical laboratories at that time. Sodium deoxycholate has been replaced by bile salts in reduced concentration. This allows growth of *Shigella* as well as the *Salmonellae*. The peptone concentrations have been increased in order to offset the inhibitory effects of the bile salts. Hektoen Enteric Agar is currently recommended as one of several plating media for the culture of Enterobacteriaceae from stool specimens. Foods containing poultry, eggs or dairy products are the most frequent vehicles for foodborne Salmonellosis, and a variety of procedures have been developed using Hektoen Enteric Agar as part of the multi-step procedure to isolate *Salmonella*. This medium is recommended by United States Pharmacopoeia, 2009 for testing the presence of *Salmonella* in dietary supplements. This medium is recommended in testing of *Salmonella* in food sample by various standards. The composition & performance criteria of this medium are as per the specifications laid down in ISO 21567:2004.

COMPOSITION

Ingredients	Gms / Ltr
Agar	15.000
Proteose peptone	12.000
Lactose	12.000
Sucrose	12.000
Bile salts mixture	9.000
Sodium chloride	5.000
Sodium thiosulphate	5.000
Yeast extract	3.000
Salicin	2.000
Ferric ammonium citrate	1.500
Acid fuchsin	0.100
Bromothymol blue	0.065

PRINCIPLE

The increased concentration of carbohydrate and proteose peptone helps to reduce the inhibitory effect of bile salts and indicators and allows good growth of *Salmonella* and *Shigella* species while inhibiting the normal intestinal flora. The medium contains three carbohydrates, i.e., lactose, sucrose and salicin for differentiation of enteric pathogens. The higher lactose concentration aids in the visualization of enteric pathogens and minimizes the problem of delayed lactose fermentation. Salicin is fermented by many coliforms including those that do not ferment lactose and sucrose. Combination of ferric ammonium citrate and sodium thiosulphate in the medium enables the detection of hydrogen sulfide production, thereby aiding in the differentiation process due to the formation of black centered colonies. The indicator system, consisting of acid fuchsin and bromothymol blue, has lower toxicity as compared to other enteric media, resulting in improved recovery of enteric pathogens. Hoben et al further enhanced the selectivity of the medium by addition of novobiocin at a concentration of 15 mg/litre, which inhibits *Citrobacter* and *Proteus* species.

INSTRUCTION FOR USE

- Dissolve 76.67 grams in 1000ml distilled water.
- Gently heat to boiling with gentle swirling and dissolve the medium completely. Do Not Autoclave.
- Cool to 45-50°C.
- Mix well and pour into sterile petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Dehydrated powder	:	Cream to yellow colour with tancast, homogeneous free flowing powder
Appearance of Prepared medium	:	Green coloured, clear to slightly opalescent gel.
pH (at 25°C)	:	7.5± 0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Colour of colony	Incubation Temperature	Incubation Period
<i>Salmonella enteritidis</i>	13076	50-100	Luxuriant	>=50%	Greenish blue, may have black centres (H ₂ S production)	35-37°C	18-24 Hours
<i>Salmonella typhi</i>	6539	50-100	Luxuriant	>=50%	Greenish blue, may have black centres (H ₂ S production)	35-37°C	18-24 Hours
<i>Salmonella typhimurium</i>	14028	50-100	Luxuriant	>=50%	Greenish blue, may have black centres (H ₂ S production)	35-37°C	18-24 Hours
<i>Shigella flexneri</i>	12022	50-100	Luxuriant	>=50%	Greenish blue	35-37°C	18-24 Hours
<i>Escherichia coli</i>	25922	50-100	Fair	20-30%	Orange (may have bile precipitate)	35-37°C	18-24 Hours
<i>Escherichia coli</i>	8739	50-100	Fair	20-30%	Orange (may have bile precipitate)	35-37°C	18-24 Hours

PACKAGING

In 100 & 500 gm packaging size.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°C and protect from direct Sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

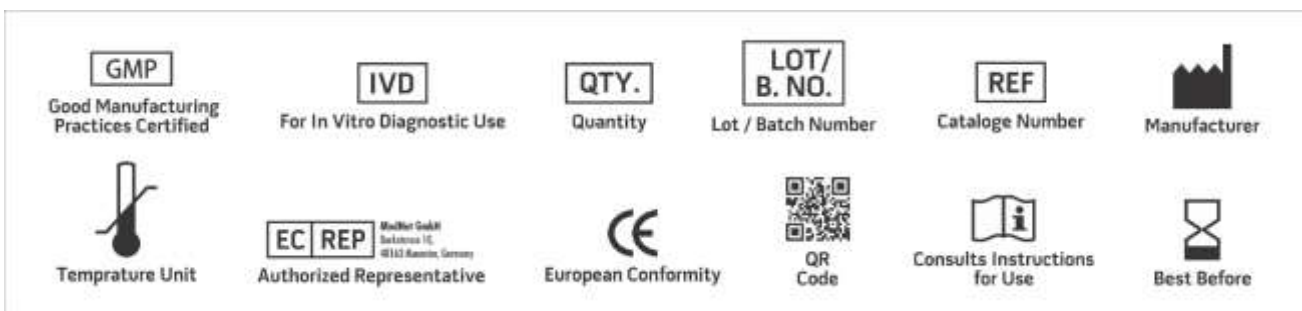
Product Deterioration: Do not use, if powder show evidence of microbial contamination, discoloration, drying, or other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**
Revision: 9th July 2020