Hektoen/Agar

356-3894
355-4386
356-4284

DEFINITION

Medium used for the isolation of *Enterobacteria* and for the differentiation of pathogenic *Enterobacteria* in the analysis of food products.

STANDARDS

FOOD MICROBIOLOGY

- **NF EN ISO 21567 (March 2005):** Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Shigella* spp.

PRINCIPLE

The principle of the medium relies on the ability or otherwise of *Enterobacteria* to ferment three sugars: lactose, saccharose and salicin.
Two indicators display the reaction: bromothymol blue, which turns yellow in the presence of acidity, and fuchsin, which colors in the presence of aldehyde.
Further differentiation relying on the production of hydrogen sulfide is possible due to the presence of sodium thiosulfate and ferric citrate.
This is manifested by colonies with a black center, coloration resulting from the formation of ferric sulfide.
Due to the presence of bile salts, this medium inhibits Gram positive bacteria.

PRESENTATION

- **Pre-poured**
  90 mm x 20 dishes — **code 356-3894**

- **Ready-to-use**
  100 ml x 6 bottles — **code 355-4386**

- **Dehydrated**
  500 g — **code 356-4284**

STORAGE

- Pre-poured: + 2°C to 20°C.
- Ready-to-use: + 2°C to 8°C.
- Dehydrated: + 15°C to 25°C, in carefully-sealed bottle in a cool, dry place.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Peptone proteose	12 g
Yeast extract	3 g
Sodium chloride	5 g
Sodium thiosulfate	5 g
Bile salts	9 g
Ferric ammonium citrate	1.5 g
Salicin	2 g
Lactose	12 g
Saccharose	12 g
Fuchsin acid	100 mg
Bromothymol blue	65 mg
Agar	13 g
Distilled water	1,000 ml

Final pH (25°C) = 7.5 ± 0.2

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- Diluent(s)
- Distilled water

EQUIPMENT REQUIRED (NOT SUPPLIED)

(non-exhaustive)

- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Mixer-homogenizer
- 100 ml bottles with autoclave-proof stoppers
- Sterile Petri dishes (Ø = 90 mm)
- Sterile Pasteur pipettes (**code 355-0751**) or inoculating loop
- Water-bath precise to ± 1°C
- Thermostatically-controlled incubator or incubating room, precise to ± 1°C
- All usual laboratory equipment

PREPARATION OF DEHYDRATED MEDIUM

Always shake well before use.
Dissolve 75 g of powder in 1 liter of distilled water. Heat gently, swirling frequently, then bring to the boil until completely dissolved.
DO NOT AUTOCLAVE.
Cool to 44°C - 49°C according to the standards, and pour on Petri dishes.

Reconstitution ratio: 75 g/l
500 g of powder makes 6.6 liters of medium.

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PROTOCOL

• Inoculation and incubation

After selective enrichment for the detection of *Salmonella*, inoculate in streaks using an inoculating loop or a Pasteur pipette loop (previously flame-sterilized).
Incubate at 37 ℃ ± 1 ℃ for 24 to 48 hours.

READING AND INTERPRETATION

- Salmon-colored colonies: *Escherichia, Levinea, Citrobacter, Klebsiella, Enterobacter, Serratia, Yersinia*.

- Salmon-colored colonies with a black center: *Proteus vulgaris.*

- Blue-green colonies with a black center: suspicion of *Salmonella* to be distinguished from *Proteus mirabilis.*

- Blue-green or green colonies: suspicion of *Shigella* or of *Salmonella* to be distinguished from *Proteus morganii* or *rettgeri from Providencia, Hafnia, Levinea, Plesiomonas*.

N.B.:

1 - Some *Salmonella arizonae* and *Shigella sonnei* produce salmon/yellow colonies.
2 - *Pseudomonas* colonies are small, brown or bluish.
3 - *Vibrio cholerae* may develop: colonies of these are pinkish-yellow.

PRECAUTIONS

- The time lapse between the end of preparation of the stock solution (or the 10^{-1} dilution in the case of a solid product) and the moment when the dilutions come into contact with the culture medium must not exceed 15 minutes.
- **DO NOT AUTOCLAVE THE MEDIUM.**
- Comply with Good Laboratory Practice.

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from reception of raw materials through to commercialisation of end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Results after 24-48h culture at 37℃
Escherichia coli ATCC 25922	Salmon-colored colonies Partial inhibition
Salmonella Enteritidis ATCC 13076	Blue-green colonies with black center
Shigella sonnei ATCC 25931	Green to salmon colonies
Shigella flexneri ATCC 12022	Green colonies
Proteus mirabilis ATCC 25933	Blue-green colonies with black center
Enterobacter aerogenes ATCC 13048	Salmon colonies
Enterococcus faecalis var zymogenes ATCC 29212	Inhibition

KEY WORDS

Hektoen / *Enterobacteria* / Food products / Isolation / Differentiation / Lactose / Saccharose / Salicin / *Salmonella* / Bromothymol blue / Fushin / Sodium thiosulfate/
Ferric citrate / Medium.

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