SS/Agar
(*Salmonella - Shigella*)

356-2717 / 356-3814
356-4514

DEFINITION
Selective medium used in the analysis of food products for the isolation and differentiation of *Salmonella* and *Shigella* after pre-enrichment.

STANDARDS
FOOD MICROBIOLOGY
- **Resolution n°134 (July 1999):** Detection of *Shigella*.

PRINCIPLE
The principle of the medium relies on the inability of *Salmonella* and *Shigella* to ferment lactose (colorless colonies). In addition, certain *Salmonella* can reduce sulfates to sulfides in the presence of ferric citrate (colonies with a black center).
Due to the presence of brilliant green and bilesalts, the medium inhibits other bacteria.

PRESENTATION
- **Pre-poured**
  20 plates x 90 mm — **code 356-3814**
- **Ready-to-use**
  200 ml x 6 bottles — **code 356-2717**
- **Dehydrated**
  500 g — **code 356-4514**

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

THEORETICAL FORMULA

Peptone	5 g
Bovine meat extract	5 g
Bile salts	4.2 g
Sodium citrate	10 g
Sodium thiosulfate	8.5 g
Ferric citrate	2 g
Lactose	10 g
Neutral red	25 mg
Brilliant green	0.3 mg
Agar	12 g
Distilled water	1,000 ml

Final pH (25°C) = 7.3 ± 0.2

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)
- Distilled water

EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)
- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Mixer-homogenizer
- Sterile Petri dishes (Ø= 90 mm)
- Sterile Pasteur pipettes (**code 355-0751**) or inoculating loops
- Water-bath precise to ± 1°C
- Thermostatically-controlled incubator or incubation room, precise to ± 1°C
- All usual laboratory equipment

PREPARATION OF DEHYDRATED MEDIUM
Always shake well before use.
Dissolve 56.7 g of powder in 1 liter of distilled water. **DO NOT AUTOCLAVE**
Bring to boiling point, swirling frequently to dissolve the agar. Cool to 44°C - 47°C.

Reconstitution ratio: 56.7 g/l.
500 g of powder makes 8.8 liters of medium.

PROTOCOL
Primary enrichment
Inoculate the following enrichment media at the same time:
- Selenite-Cystine medium
- Müller-Kauffman medium (**code 356-9334**) or Rappaport-Vassiliadis (RVS) medium (**code 356-4324**) or Hektoen medium (**code 356 4284**).

Incubate at 37°C ± 1°C for 24 hours.

(*See corresponding Technical Sheets*).

Inoculation and incubation
After incubation, sub-culture the 2 enrichment media on plates of SS agar. Incubate at 37°C ± 1°C for 24-48 hours.
If no colonies develop on any of the plates after this period of incubation, the isolation on enrichment media should be repeated.

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READING AND INTERPRETATION
After 48 hours incubation, colonies usually present the typical appearance shown below (lactose-positive and lactose-negative colonies, however, can be readily differentiated after only 24 hours incubation)

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.**
- Avoid a drop in volume of the medium through evaporation during heating. If necessary, make up the volume with water.
- Comply with Good Laboratory Practice.

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°C
Escherichia coli ATCC 25922	Partial inhibition Pink to red colonies
Salmonella Enteritidis ATCC 13076	Colonies with a black centre
Shigella sonnei ATCC 25931	Pink to red colonies
Proteus mirabilis ATCC 25933	Colonies with a black centre
Enterobacter aerogenes ATCC 13048	Pink to red colonies
Enterococcus faecalis var zymogenes ATCC 29212	Inhibition

QUALITY CONTROL OF MANUFACTURER
Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the 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.

KEY WORDS
Salmonella-Shigella / Food products / Isolation / Pre-enrichment / Lactose / Medium.

BIBLIOGRAPHY

- **ISENBERG H.D., KOMINOS S. and SIEGEL M. (1969):** Isolation of *Salmonellae* and *Shigellae* from an artificial mixture of fecal bacteria. Applied Microbiology 18 (4): 656.

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