

## RAPPAPORT VASSILIADIS BROTH (ISO)

RSVB-00I-500

- **Principle**

Rappaport Soy Broth (Vassiliadis) is recommended by ISO 6579 and ISO 19250, after the preenrichment step, for the selective isolation of *Salmonella* spp.

Rappaport medium was modified by Vassiliadis by reducing Malachite green concentration and increasing incubation temperature, thus offering a better stability of the pH of the prepared medium and optimizing the concentration of Magnesium chloride, resulting in an improved recovery of Salmonellae.

Soy peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Potassium phosphates balance the low pH of the medium, combined with the presence of Magnesium chloride to raise the osmotic pressure, and Malachite green to inhibit other organisms. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

- **Regulatory compliance**

This product is manufactured under a quality management system in accordance with ISO 9001 and ISO 13485, and its formulation and quality control comply with applicable international standards, such as ISO 11133, where relevant.

For this specific medium, compliance is also established with the relevant requirements of ISO 19250 and ISO 6579.

- **Composition**

Ingredients	g/L
Dipotassium phosphate	0.18
Malachite green	0.036
Sodium chloride	7.20
Magnesium chloride anhydrous	13.40
Monopotassium phosphate	1.26
Soy peptone	4.50

- **Preparation**

Suspend 26.6 grams of the medium in 1 litre of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 115 °C for 15 minutes. DO NOT OVERHEAT.

- **Applications and use**

\* For detection of *Salmonella* spp. in food, animal feed, animal faeces, and environmental samples according to ISO 6579:

- Preenrichment in non-selective liquid medium: Inoculate the Buffered Peptone Water (AGPT-ODI-500) with the sample or dilutions and incubate at 34-38 °C for 18±2 h.
- Enrichment in/on selective media: Inoculate, with the culture obtained in the pre-enrichment stage, the Rappaport Soy Broth (Vassiliadis) and the MKKTN Broth (MKFB-00I-500). The Rappaport Soy Broth and the Modified Semisolid Rappaport medium are incubated at 41.5 °C for 24±3 h, and the MKKTN Broth at 34-38 °C for 24±3 h.
- Plating out on selective solid media: From the selective enriched cultures, inoculate two selective isolation agars; XLD agar (AGXL-00I-500) and any other selective medium complementary to XLD agar, such as Brilliant Green Agar (BGAG-00I-500), Hektoen Enteric Agar (HEAG-00P-500) and Salmonella Shigella Agar (SSAG-IEP-500). Incubate the XLD plates inverted at 34-38 °C for 24±3 h. Incubate the second selective medium in accordance with the manufacturer's instructions.
- Confirmation: Subculture colonies of presumptive Salmonella and confirm their identity by biochemicals and serological tests.

Note: According to Annex D of ISO 6579-1: 2017, for the detection of enterica subspecies enterica serovars Typhi and Paratiphy, Selenite Cystine Broth should be added as a selective enrichment medium, and Bismuth Sulphite Agar (Wilson Blair) should be selected as a second selective medium.

\* For detection of *Salmonella* spp. in water samples according to ISO 19250:

- Preenrichment in non-selective medium: Inoculate the Buffered Peptone Water (AGPT-ODI-500) with the sample or dilutions and incubate at 34-38 °C for 18±2 h.
- Enrichment in selective media: Inoculate, with the culture obtained in the pre-enrichment stage, the Rappaport Soy Broth (Vassiliadis)(RSVB-00I-500) and the MKKTN Broth. The Rappaport Soy Broth is incubated at 41.5±1 °C and the MKKTN Broth at 34-38 °C, both for 24±3 hours.
- Plating out on selective solid media: From the selective enriched cultures, inoculate two selective isolation agars; XLD agar (AGXL-00I-500) and any other selective medium complementary to XLD agar (For instance, Brilliant Green Agar (BGAG-00I-500)). Incubate the XLD plates inverted at 34-38 °C for 24±3 hours. Incubate the second selective medium in accordance with the manufacturer's instructions.
- Confirmation: Subculture colonies of presumptive Salmonella and confirm their identity by biochemicals and serological tests.

- **Quality control**

<b>Solubility</b>	w/o rests
<b>Appearance</b>	Fine powder
<b>Colour of the dehydrated medium</b>	Beige
<b>Colour of the prepared medium</b>	Blue
<b>Final pH (25 °C)</b>	5.2 ± 0.2

- **Microbiological test**

According to ISO 11133:

Incubation conditions: 41.5±1 °C / 24±3 h.

Inoculation conditions: Productivity qualitative (<100 CFU) / Selectivity (10<sup>4</sup>-10<sup>6</sup> CFU).

Microorganisms	ATCC	Specification	Characteristic reaction
<i>Salmonella typhimurium</i> + <i>Escherichia coli</i> + <i>Pseudomonas aeruginosa</i>	14028 + 8739 + 27853	> 10 colonies in XLD or another chosen medium	Colonies with black centre and a lightly transparent zone of reddish colour due to the colour change of the medium
<i>Salmonella enteritidis</i> + <i>Escherichia coli</i> + <i>Pseudomonas aeruginosa</i>	13076 + 8739 + 27853	> 10 colonies in XLD or another chosen medium	Colonies with black centre and a lightly transparent zone of reddish colour due to the colour change of the medium
<i>Enterococcus faecalis</i>	29212	< 10 colonies in TSA	-
<i>Escherichia coli</i>	8739	Partial inhibition <100 colonies in TSA	-

- **Storage**

The product is highly hygroscopic; keep the container always closed and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Temp. Min.:2 °C Temp. Max.:25 °C.

Note: Sterilize media immediately after reconstitution.

- **Bibliography**

Rappaport F., Konforti N. and Navon B. (1 956) J. Clin Pathol., 9,261.

Peterz M. Wiberg C. and Norberg P. (1989) J. Appl. Bact. 66: 523-528.

UNE-EN-ISO 6579. Food Microbiology for human consumption and Animal Feed. Horizontal Method for the detection of *Salmonella* spp.

ISO19250 Water Quality-Detection of *Salmonella* spp.

ISO 6579 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSR and SC.

- **Product use limitation**

This product is developed, designed and supplied exclusively for research use only. It is not intended for diagnostic applications or drug development, and it is not suitable for administration to humans or animals.