Technical Data Sheet

GranuCult® prime
RAPPAPORT-VASSILIADIS Salmonella Enrichment broth
acc. Ph. Eur./USP/JP and ChP

Ordering number: 1076660500

For the selective enrichment of *Salmonella* spp. from pharmaceutical and other materials.

RAPPAPORT-VASSILIADIS Salmonella Enrichment broth acc. Ph. Eur./USP/JP and ChP is also known as RVSEB, Rappaport Vassiliadis Salmonella broth and RVS broth.

This culture medium complies with the specifications given by the harmonized methods of Ph. Eur./USP/JP: Ph. Eur. chapter 2.6.13, USP chapter <62>, JP chapter 4.05 I as well as USP chapter <2022> and ChP chapter 1106.

The performance test of this culture medium complies with the current versions of the harmonized methods of Ph. Eur./USP/JP: Ph. Eur. chapter 2.6.13, USP chapter <62>, JP chapter 4.05-II.

Mode of Action

A modification of the originally described Rappaport medium, using soya peptone instead of tryptone (peptone from casein) has been reported to improve *Salmonella* recovery rates (Van Schothorst and Renaud, 1983 and Van Schothorst et al., 1987). This is the composition of Rappaport-Vassiliadis Salmonella enrichment broth used in various pharmacopoeias.

The efficiency of RVS broth for enrichment of *Salmonella* is based on their ability to multiply at relatively high osmotic pressures, relatively low pH values, high temperature, relatively high resistance to malachite green and modest nutritional requirements. Magnesium chloride and malachite green inhibit the growth of the accompanying bacterial flora. This selectivity is increased by the hypertonic effect of magnesium chloride in favor of supporting *Salmonella* growth.

Other selective enrichment and isolation media may be needed for the detection of some *Salmonella* serovars, e.g. for the detection of *Salmonella enterica* subspecies *enterica* serovars Typhi and Paratyphi.

Typical Composition

Specified by Ph. Eur. 2.6.13, USP <62> and <2022>, JP 4.05-II, ChP 1106		GranuCult® prime RAPPAPORT-VASSILIADIS Salmonella Enrichment broth acc. Ph. Eur./USP/JP and ChP	
Soya peptone	4.5 g/l	Soya peptone	4.5 g/l
Sodium chloride	8.0 g/l	Sodium chloride	8.0 g/l
di-Potassium hydrogen phosphate	0.4 g/l	di-Potassium hydrogen phosphate	0.4 g/l
Potassium dihydrogen phosphate	0.6 g/l	Potassium dihydrogen phosphate	0.6 g/l
Magnesium chloride hexahydrate	29.0 g/l	Magnesium chloride anhydrous (equivalent to 29,0 g/l magnesium chloride hexahydrate)	13,6 g/l
Malachite green	0.036 g/l	Malachite green	0.036 g/l
Water	1000 ml/l	Water	n/a
pH at 25 °C	5.2 ± 0.2	pH at 25 °C	5.2 ± 0.2

Preparation

Dissolve 27.1 g in 1 liter of purified water. Dispense into tubes or flasks and autoclave (15 minutes at 115 °C).

The dehydrated medium is a granulate with blue green colour.

The prepared medium is clear, possibly with a slight precipitate, and darkblue.

The pH value at 25 °C is in the range of 5.2 ± 0.2.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Allow the RAPPAPORT-VASSILIADIS Salmonella Enrichment broth to equilibrate at room temperature if it was stored at a lower temperature.

According to Ph. Eur., USP, JP and ChP, transfer 0.1 ml of the culture obtained in casein soya bean digest broth (TSB) to a tube containing 10 ml of RAPPAPORT-VASSILIADIS Salmonella Enrichment broth.

Incubate the inoculated broth under aerobic conditions at (30 - 35) °C for (18 - 24) h.

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Subculture on plates of Xylose, lysine deoxycholate (XLD) agar and incubate at (30 - 35) °C for (18 – 48) h.

According to USP <2022>, subculture additionally on Brilliant green agar and Hektoen enteric agar and incubate all subcultures at (30 - 35) °C for (24 – 48) h.

On Xylose, lysine deoxycholate agar, the possible presence of *Salmonella* is indicated by the growth of well-developed, red colonies, with or without black centers.

According to Ph. Eur., USP, JP and ChP, this is confirmed by identification tests.

Storage

Store at +15 °C to +25 °C, dry and tightly closed. Do not use clumped or discolored medium. Protect from UV light (including sun light). For *in vitro* use only.

Microbiological Performance

The performance test complies current versions of the harmonized methods of Ph. Eur./USP/JP: Ph. Eur. chapter 2.6.13, USP chapter <62>, JP chapter 4.05-II as well as USP chapter <2022>.

Control strains	Inoculum	Incubation	Growth	Specified by
Salmonella enterica serovar Typhimurium ATCC® 14028™ [WDCM 00031]	10 - 100 CFU	not more than 18 hours at 30 – 35 °C, aerobic	+	Ph. Eur. 2.6.13, USP <62> and <2022>, JP chapter 4.05-II
Salmonella enterica serovar Abony NCTC® 6017™ [WDCM 00029]	10 - 100 CFU	not more than 18 hours at 30 – 35 °C, aerobic	+	
Staphylococcus aureus ATCC® 6538™ [WDCM 00032]	≥ 100 CFU	less than 24 hours at 30 – 35 °C, aerobic	No growth	

Please refer to the actual batch related Certificate of Analysis.

Reference medium (inoculum): Tryptic Soy Agar.

Literature

European Directorate for the Quality of Medicines and Healthcare. (2023): The European Pharmacopoeia (Ph. Eur.). 11th Ed. Chapter 2.6.13 Microbiological examination of non-sterile products: Test for specified products. Strasbourg, France.

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Japanese Ministry of Health, Labor and Welfare. (2021): The Japanese Pharmacopoeia (JP). 18th Ed. Chapter 4.05 Microbial Limit Test-II. Microbiological examination of non-sterile products: Test for specified products. Japanese Ministry of Health, Labor and Welfare. Tokyo, Japan.

Pharmacopoeia of the People's Republic of China (ChP). (2020): Section 1100 Biological Tests. Chapter 1106 Microbiological examination of nonsterile products: Test for specified microorganisms. People's Medical Publishing House, Beijing, China.

United States Pharmacopeial Convention. (2024): The United States Pharmacopeia (USP). Chapter ⟨62⟩ Microbiological examination of nonsterile products: Test for specified micro-organisms and Chapter ⟨2022⟩ Microbiological procedures for absence of specified microorganisms – Nutritional and dietary supplements. Rockville, Md., USA.

Peterz M., Wiberg, C. and Norberg, P. (1989) The effect of incubation temperature and magnesium chloride concentration on growth of *salmonella* in home-made and in commercially available dehydrated Rappaport-Vassiliadis broths. J Appl. Bact. **66**: 523-528.

Rappaport, F., Konforti, N. and Navion, B. (1956): A new enrichment medium for certain salmonellae. J. Clin. Pathol. **9**, 261-266.

Roesti, D. (2020): Microbiological examination of non - sterile final dosage forms and raw material including acceptance criteria and testing frequency. In: Pharmaceutical Microbiological Quality Assurance and Control: Practical Guide for Non-Sterile Manufacturing. (Roesti, D. and Goverde, M. eds.)., pp. 105-151. John Wiley & Sons, Inc., Hoboken, NJ, USA.

van Schothorst M. and Renaud, A.M. (1983): Dynamics of salmonellae isolation with modified Rappaport's medium (R 10). J. Appl. Bact. **54**: 209-215.

Ordering Information

Product	Cat. No.	Pack size
GranuCult® prime RAPPAPORT-VASSILIADIS Salmonella Enrichment broth acc. Ph. Eur./USP/JP and ChP	1076660500	500 g
GranuCult® prime Tryptic Soy Broth Casein-peptone soymeal-peptone broth for microbiology (According harm. EP/USP/JP and ISO)	1054590500	500 g
GranuCult® prime XLD Agar (acc. harm. EP/USP/JP)	1052900500	500 g

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