

Technical Data Sheet

GranuCult® prime

RVS (RAPPAPORT-VASSILIADIS-Soya) broth

acc. ISO 6579, ISO 19250, EN 15215 and USDA-FSIS

Ordering number: 1077000500

For the selective enrichment of *Salmonella* spp. from products intended for human consumption and for the feeding of animals, environmental samples in the area of food production and food handling, samples from the primary production stage, water, sludge and other materials.

RVS (RAPPAPORT-VASSILIADIS-Soya) broth acc. ISO 6579, ISO 19250, EN 15215 and USDA-FSIS is also known as Rappaport-Vassiliadis enrichment medium.

This culture medium complies with the specifications given by EN ISO 6579-1:2017/Amd 1:2020, EN ISO 19250:2010, CEN/TR 15215-2:2006, CEN/TR 15215-3:2006, USDA-FSIS MLG 1.14:2024 and GB 4789.4-2024.

The performance test of this culture medium complies with the current versions of EN ISO 11133 and EN ISO 6579-1.

This culture medium is released by the quality control laboratory of Merck KGaA, Darmstadt, Germany. The laboratory is accredited by the German accreditation authority DAkkS as registered test laboratory D-PL-15185-01-00 according to DIN EN ISO/IEC 17025 for the performance testing of media for microbiology according to DIN EN ISO 11133.

Mode of Action

A modification of the originally described Rappaport medium, using soya peptone instead of tryptone (peptone from casein) was reported to improve recovery rates of *Salmonella* (van Schothorst and Renaud, 1983 and van Schothorst et al., 1987). This is in use as Rappaport-Vassiliadis soya peptone (RVS) broth.

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. For the detection of *Salmonella enterica* subspecies *enterica* serovars Typhi and Paratyphi, the procedure as described by EN ISO 6579-1:2017/Amd 1:2020 uses additional steps – enrichment in Selenite cystine (SC) medium and plating out on Bismuth sulphite (BS) agar.

Typical Composition

Specified by EN ISO 6579-1/Amd 1:2020, EN ISO 19250:2010, CEN/TR 15215-2:2006, CEN/TR 15215-3:2006		Specified by USDA-FSIS MLG 1.14:2024, GB 4789-4:2024		GranuCult® prime RVS (RAPPAPORT-VASSILIADIS- Soya) broth acc. ISO 6579, ISO 19250, EN 15215 and USDA-FSIS		
Enzymatic digest of soya**	4,5 g/l	Peptone from soymeal	4,5 g/l	Enzymatic digest of soya	4,5 g/l	
Sodium chloride	7,2 g/l	Sodium chloride	7,2 g/l	Sodium chloride	7,2 g/l	
Potassium dihydrogen phosphate (KH ₂ PO ₄ + K ₂ HPO ₄)	1,44 g/l	di-Potassium hydrogen phosphate	0,18 g/l	di-Potassium hydrogen phosphate	0,18 g/l	1,44 g/l
		Potassium dihydrogen phosphate	1,26 g/l	Potassium dihydrogen phosphate	1,26 g/l	
Magnesium chloride (MgCl ₂), anhydrous**	13,4 g/l	Magnesium chloride, anhydrous**	13,4 g/l	Magnesium chloride anhydrous (equivalent to 28,6 g/l magnesium chloride hexahydrate)	13,4	
Malachite green oxalate	0,036 g/l	Malachite green oxalate	0,036 g/l	Malachite green oxalate	0,036 g/l	
Water	1000 ml/l	Water	1000 ml	Water	n/a	
pH at 20 – 25 °C	5.2 ± 0.2	pH at 25 °C	5.2 ± 0.2	pH at 25 °C	5,2 ± 0.2	

* CEN/TR 15215-2:2006 and CEN/TR 15215-3:2006 specify a mixture of trypsin digested peptone from casein and trypsin digested peptone from meat.

** EN ISO 6579-1:2017/Amd 1:2020 specifies: Anhydrous magnesium chloride (MgCl₂) 13,4 g/l or magnesium chloride hexahydrate (MgCl₂·6H₂O) 28,6 g/l.
EN ISO 19250:2010 specifies: A mass of 28,6 g MgCl₂·6H₂O corresponds to 13,4 g MgCl₂ (anhydrous).
USDA-FSIS MLG 1.14:2024 specifies magnesium chloride hexahydrate 29 g/l. This corresponds to 13,4 g/l magnesium chloride anhydrous.
GB 4789-4:2024 specifies: Magnesium chloride (containing 6 crystal waters) 28,6 g/l. This is equivalent to magnesium chloride anhydrous 13,4 g/l.

Preparation

Dissolve 26.6 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 RVS broth to equilibrate at room temperature if it was stored at a lower temperature.

According to EN ISO 6579, transfer 0.1 ml of the culture obtained in the pre-enrichment (Buffered Peptone Water) to a tube containing 10 ml of RVS broth. Minimize the transfer of particulate material from the pre-enrichment into the selective enrichment medium.

Incubate the inoculated broth under aerobic conditions, e.g., according to EN ISO 6579 at $(41,5 \pm 1) ^\circ\text{C}$ for (24 ± 3) h. Care should be taken that the maximum allowed temperature ($42.5 ^\circ\text{C}$) is not exceeded.

From the culture obtained in RVS broth selective solid media are inoculated, see details given by EN ISO 6579 or other appropriate standard.

According to EN ISO 6579-1, for some products it may be necessary to incubate the selective enrichment medium for an additional 24 h, then follow the same plating out procedure as described above.

According to EN ISO 6579-1, it is permissible to store the selective enrichment after incubation at $(5 \pm 3) ^\circ\text{C}$ for a maximum of 72 h.

From the selective enriched cultures, inoculate selective isolation media according to the specific standard.

Storage

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

According to EN ISO 6579-1, self-prepared medium can be stored in closed containers $+2$ to $+8 ^\circ\text{C}$ in the dark and protected against evaporation for up to three months.

Microbiological Performance

The performance test complies with the current versions of EN ISO 11133 and EN ISO 6579-1.

Test method: Qualitative single tube method for selective liquid enrichment media with a mixture of target and non-target microorganisms in the same tube				
Control strains	Inoculum	Incubation	Criteria and expected results	Specified by
<i>Salmonella enterica</i> serovar Typhimurium ATCC® 14028™ [WDCM 00031] + <i>Escherichia coli</i> ATCC® 25922™ [WDCM 00013] + <i>Pseudomonas aeruginosa</i> ATCC® 27853™ [WDCM 00025]	≤ 100 CFU $\geq 10\ 000$ CFU $\geq 10\ 000$ CFU	(24 ± 3) h/ (41,5 ± 1) °C aerobic	> 10 colonies with black center on XLD agar	EN ISO 6579-1:2017/ Amd 1:2020 and EN ISO 11133:2014/ Amd 1:2018/ Amd 2:2020
<i>Salmonella enterica</i> serovar Enteritidis ATCC® 13076™ [WDCM 00030] + <i>Escherichia coli</i> ATCC® 8739™ [WDCM 00012] + <i>Pseudomonas aeruginosa</i> ATCC® 27853™ [WDCM 00025]	≤ 100 CFU $\geq 10\ 000$ CFU $\geq 10\ 000$ CFU			

Test method: Qualitative single tube method for selective liquid enrichment media with non-target microorganisms

Control strains	Inoculum	Incubation	Criteria and expected results	Specified by
<i>Escherichia coli</i> ATCC® 8739™ [WDCM 00012]	≥ 10 000 CFU	(24 ± 3) h/ (41,5 ± 1) °C aerobic	Partial inhibition, ≤ 100 colonies on Tryptic Soy Agar (TSA)	EN ISO 6579-1:2017/ Amd 1:2020 and EN ISO 11133:2014/ Amd 1:2018/ Amd 2:2020
<i>Escherichia coli</i> ATCC® 25922™ [WDCM 00013]	≥ 10 000 CFU			
<i>Enterococcus faecalis</i> ATCC® 19433™ [WDCM 00009]	≥ 10 000 CFU		< 10 colonies on Tryptic Soy Agar (TSA)	
<i>Enterococcus faecalis</i> ATCC® 29212™ [WDCM 00087]	≥ 10 000 CFU			

Please refer to the actual batch related Certificate of Analysis.

Reference medium (inoculum): Tryptic Soy Agar.

Confirmation medium: XLD agar resp. Tryptic Soy Agar.

Tryptic soy agar (TSA) is another term of Tryptone soy agar (TSA) according EN ISO 11133.

Literature

CEN European Committee for Standardization. Characterization of sludges - Detection and enumeration of *Salmonella* spp. in sludges, soils, soil improvers, growing media and biowastes - Part 2: Liquid enrichment method in selenite-cystine medium followed by Rapport-Vassiliadis for semiquantitative Most Probable Number (MPN) determination. CEN/TR 15215-2:2006.

CEN European Committee for Standardization. Characterization of sludges - Detection and enumeration of *Salmonella* spp. in sludges, soils, soil improvers, growing media and biowastes - Part 3: Presence/absence method by liquid enrichment in peptone-novobiocin medium followed by Rapport-Vassiliadis. CEN/TR 15215-3:2006.

EN ISO International Standardisation Organisation. Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of *Salmonella* — Part 1: Detection of *Salmonella* spp. + Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC. EN ISO 6579-1:2017/Amd 1:2020.

EN ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media + Amendment 1 + Amendment 2. EN ISO 11133:2014/Amd 1:2018/Amd 2:2020.

EN ISO International Standardisation Organisation. Water quality — Detection of *Salmonella* spp. EN ISO 19250:2010.

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USDA-FSIS United States Department of Agriculture - Food Safety and Inspection Service. Microbiology Laboratory Guidebook (MLG). Media and Reagents. RVS broth. MLG Appendix 1.14 (16-12-2024).

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Ordering Information

Product	Cat. No.	Pack size
GranuCult® prime RVS (RAPPAORT-VASSILIADIS-Soya) broth acc. ISO 6579, ISO 19250, EN 15215 and USDA-FSIS	1077000500	500 g

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