Technical Data Sheet

GranuCult® prime
mTSB (modified Tryptic Soy Broth) acc. USDA-FSIS

Ordering number: 1.40011.0500 / 1.40011.9010

For the selective enrichment of *Salmonella* spp. in different food and environmental samples and Top Seven Shiga Toxin-Producing *Escherichia coli* (STEC) in meat products, carcass, and environmental sponges.

This culture medium complies with the specifications given by USDA-FSIS MLG 1.14:2024.

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

This medium contains pancreatic digest of casein, papaic digest of soybean meal and casamino acids (casein acid hydrolysate) which provide carbon and nitrogen sources, vitamins and minerals, supported by glucose as carbon energy source for rapid growth during the enrichment. Sodium chloride maintains the osmotic balance and dipotassium hydrogen phosphate acts as buffering agent for maintaining the pH balance of the medium. Bile salts ensure the selectivity.

Typical Composition

Specified by USDA-FSIS MLG 1.14			GranuCult® prime mTSB (modified Tryptic Soy Broth) acc. USDA-FSIS	
Modified Tryptone Soya Broth	Pancreatic digest of casein	17.0 g/l	Pancreatic digest of casein	17.0 g/l
	Papaic digest of soybean meal	3.0 g/l	Papaic digest of soybean meal	3.0 g/l
	Sodium chloride	5.0 g/l	Sodium chloride	5.0 g/l
	Glucose	2.5 g/l	D(+)Glucose	2.5 g/l
	di-Potassium hydrogen phosphate	4.0 g/l	di-Potassium hydrogen phosphate	4.0 g/l
	Bile salts	1.5 g/l	Bile salts	1.5 g/l
Casamino acids (casein acid hydrolysate)		10.0 g/l	Casamino acids (casein acid hydrolysate)	10.0 g/l
Water		1000 ml/l	Water	n/a
pH at 25 °C		7.4 ± 0.2	pH at 25 °C	7.4 ± 0.2

Preparation

Dissolve 43.0 g in 1 liter of purified water. Dispense into suitable vessels and autoclave (15 minutes at 121°C).

The dehydrated medium is a granulate with yellow-brown color.

The prepared medium is clear to slightly opalescent and yellowish-brown.

The pH value at 25 °C is in the range of 7.4 ± 0.2.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Following the procedure given by USDA-FSIS for meat products and for carcass and environmental sponges for detection, isolation, and identification of Top Seven Shiga Toxin-Producing *Escherichia coli* (STEC), prepare for meat products in a sterile strainer bag a single sample in mTSB with a 1:4 dilution (one portion product in three portions of mTSB), e.g. 325 ± 32,5 g sample with 975 ± 19,5 ml mTSB broth. Pummel, blend or hand massage until clumps are dispersed.

For environmental and carcass sponges with 10 ml of buffer, add 50 ± 5 ml of mTSB broth. For carcass sponges with more buffer, use a 1:6 ratio of mTSB (for example, a sponge with 25 ml of buffer will use 125 ml of enrichment broth) to each sponge sample. Pummel, blend or hand massage until well mixed. Incubate all bags with their contents at (42 ± 1) °C for 15 h to 24 h. After incubation is complete, follow the procedure as given by USDA-FSIS.

Following the procedure given by USDA-FSIS for detection of *Salmonellae* from raw meat and raw beef mixed products, meat carcasses and environmental sponges, add the mTSB at a 1:4 dilution, e.g., (325 ± 32.5) g sample with (975 ± 19.5) mL mTSB broth. Stomach, or hand massage until clumps are dispersed.

For meat carcass or environmental sponges, add 50 mL of mTSB to the sample bag containing a sponge moistened with 10 mL. Mix well.

Incubate all bags with their content at (42 ± 1) °C for 15 h to 24 h. After incubation is complete, follow the procedure as given by USDA-FSIS.

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 is in accordance with the current version of EN ISO 11133.

Qualitative single tube method (turbidity) for performance testing of liquid media						
Function	**Control strains**	**Inoculum**	**Incubation**	**Method of control**	**Expected results**	**Specified by**
Productivity	*Escherichia coli* Serogroup O26:H4 ATCC® BAA-2212™	≤ 100 CFU	(18 ± 3) h / (42 ± 1) °C aerobic	qualitative	good to very good	USDA-FSIS MLG 5C.04: 2024
	Escherichia coli Serogroup O45:H10 ATCC® BAA-2649™	≤ 100 CFU			good to very good	USDA-FSIS MLG 5C.04: 2024
	Escherichia coli Serogroup O103:Nonmotile ATCC® BAA-2214™	≤ 100 CFU			good to very good	USDA-FSIS MLG 5C.04: 2024
	Escherichia coli Serogroup 111a,11b:K58:H21 ATCC® 29552™	≤ 100 CFU			good to very good	USDA-FSIS MLG 5C.04: 2024
	Escherichia coli Serogroup O121:Nonmotile ATCC® BAA-2190™	≤ 100 CFU			good to very good	USDA-FSIS MLG 5C.04: 2024
	Escherichia coli Serogroup O145:H34 ATCC® BAA-2216™	≤ 100 CFU			good to very good	USDA-FSIS MLG 5C.04: 2024

Qualitative single tube method for selective liquid enrichment media with a mixture of target and non-target microorganisms in the same tube					
Function	**Control strains**	**Inoculum**	**Incubation**	**Method of control**	**Expected results**
Productivity	*Salmonella* Typhimurium ATCC® 14028™ [WDCM 00031] + *Staphylococcus aureus* ATCC® 25923™ [WDCM 00034]	≤ 100 CFU ≥ 10^4 CFU	(18 ± 3) h / (42 ± 1) °C aerobic	qualitative	> 10 red colonies on Rambach® agar
	Salmonella Enteritidis ATCC® 13076™ [WDCM 00030] + *Staphylococcus aureus* ATCC® 25923™ [WDCM 00034]	≤ 100 CFU ≥ 10^4 CFU			> 10 red colonies on Rambach® agar
	Salmonella Abaetetuba ATCC® 35640™ + *Staphylococcus aureus* ATCC® 25923™ [WDCM 00034]	≤ 100 CFU ≥ 10^4 CFU			> 10 red colonies on Rambach® agar
	Salmonella Cholerasuis ATCC® 13312™ + *Staphylococcus aureus* ATCC® 25923™ [WDCM 00034]	≤ 100 CFU ≥ 10^4 CFU			> 10 red colonies on Rambach® agar

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Qualitative single tube method for selective liquid enrichment media with a mixture of target and non-target microorganisms in the same tube					
Function	**Control strains**	**Inoculum**	**Incubation**	**Method of control**	**Expected results**
Productivity	*Escherichia coli* Serogroup O157:H7 ATCC® 35150™ + *Staphylococcus aureus* ATCC® 25923™ [WDCM 00034]	≤ 100 CFU ≥ 10^4 CFU	(18 ± 3) h / (42 ± 1) °C aerobic	qualitative	> 10 transparent small colonies with a pale yellowish-brown appearance on SMAC agar*
	Escherichia coli Serogroup O157:H7 ATCC® 700728™ [WDCM 00014] + *Staphylococcus aureus* ATCC® 25923™ [WDCM 00034]	≤ 100 CFU ≥ 10^4 CFU			> 10 transparent small colonies with a pale yellowish-brown appearance on SMAC agar
	Escherichia coli ATCC® 25922 [WDCM 00013] + *Staphylococcus aureus* ATCC® 25923™ [WDCM 00034]	≤ 100 CFU ≥ 10^4 CFU			no limit; if growth: pink colonies on SMAC agar

Qualitative single tube method for selective liquid enrichment media with non-target microorganisms					
Function	**Control strains**	**Inoculum**	**Incubation**	**Method of control**	**Expected results**
Selectivity	*Staphylococcus aureus* ATCC® 25923™ [WDCM 00034]	≥ 10^4 CFU	(18 ± 3) h / (42 ± 1) °C aerobic	qualitative	< 10 colonies on Tryptic Soy Agar (TSA)

* SMAC Agar: Sorbitol MacConkey Agar

Please refer to the actual batch related Certificate of Analysis.

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Literature

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.

USDA-FSIS United States Department of Agriculture – Food Safety and Inspection Service. Laboratory Guidebook Chapter MLG 4.15 (2024): Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, Siluriformes (Fish) Products and Carcass and Environmental Sponges. Washington D.C., USA.

USDA-FSIS United States Department of Agriculture – Food Safety and Inspection Service. Laboratory Guidebook Chapter MLG 5C.04 (2024): Detection, Isolation, and Identification of Top Seven Shiga Toxin-Producing *Escherichia coli* (STEC) from Meat Products, Carcass, and Environmental Sponges. Washington D.C., USA.

USDA-FSIS United States Department of Agriculture – Food Safety and Inspection Service. Laboratory Guidebook Chapter MLG Appendix 1.14 (2024) Media and Reagents. Washington D.C., USA.

Ordering Information

Product	Cat. No.	Pack size
GranuCult® prime mTSB (modified Tryptic Soy Broth) acc. USDA-FSIS	1.40011.0500	500 g
GranuCult® prime mTSB (modified Tryptic Soy Broth) acc. USDA-FSIS	1.40011.9010	10 KG
NutriSelect® prime Sorbitol-MacConkey Agar (SMAC Agar) acc. ISO 16654 and FDA-BAM	1.00213.0500	500 g
Chromocult® Rambach® agar ref. ISO 6579	1.00188.0002	Kit for 4 x 250 ml
Chromocult® Rambach® agar ref. ISO 6579	1.00188.0004	Kit for 4 x 1000 ml
GranuCult® Tryptic Soy Agar acc. EP, USP, JP, ISO and FDA-BAM	1.05458.0500	500 g

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