Technical Data Sheet

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

Ordering number: 1.40011.0500 / 140011.9010

For the selective enrichment of *Salmonella* spp., *Escherichia coli* O157:H7 and non-O157 Shiga Toxin-Producing *E. coli* (STEC) in foods and environmental samples.
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

mTSB (Modified Tryptone Soya Broth) without addition of novobiocin is given by USDA-FSIS Laboratory Guidebook for the selective enrichment of non-O157 Shiga Toxin-Producing *Escherichia coli* (STEC) from meat products and from carcass and environmental sponges.

USDA-FSIS Laboratory Guidebook specifies mTSB+n (Modified Tryptone Soya Broth with Novobiocin) for outbreak-related samples of fermented sausage or cooked meat products for the selective enrichment for detection of *Escherichia coli* O157:H7.

For the isolation of *Salmonellae* from meat carcass and environmental sponges, USDA-FSIS Laboratory Guidebook specifies mTSB (without addition of novobiocin).

Pancreatic digest of casein, papaic digest of soybean meal and casamino acids (casein acid hydrolysate) are providing 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 as well as novobiocin which is added separately to form mTSB+n when required.

Typical Composition

Specified by USDA-FSIS		GranuCult® mTSB (modified Tryptic Soy Broth) acc. USDA-FSIS	
Pancreatic digest of casein*	17 g/l	Pancreatic digest of casein	17 g/l
Papaic digest of soybean meal*	3 g/l	Papaic digest of soybean meal	3 g/l
Sodium chloride*	5 g/l	Sodium chloride	5 g/l
Glucose*	2,5 g/l	D(+)Glucose	2,5 g/l
di-Potassium hydrogen phosphate*	4 g/l	di-Potassium hydrogen phosphate	4 g/l
Bile salts*	1,5 g/l	Bile salts*	1,5 g/l
Casamino acids (casein acid hydrolysate)	10 g/l	Casamino acids (casein acid hydrolysate)	10 g/l
Water	1000 ml/l	Water	n/a
Supplement after autoclaving – only for mTSB with Novobiocin (mTSB+n):			
Sodium novobiocin solution (concentration of 4 mg/ml)	2 ml	Novobiocin sodium salt	8 mg/l
pH at 25 °C	7.4 ± 0.2	pH at 25 °C	7.4 ± 0.2

* Specified by USDA-FSIS as an ingredient of Modified Tryptone Soya Broth 33 g/l.

Preparation

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

For mTSB with Novobiocin, add to 1 litre of autoclaved mTSB the content of 1 vial mTSB selective supplement (article number 120593), dissolved in 1 ml of sterile purified water. Add to the broth after it has cooled below 50°C. The supplement is homogeneously distributed in the broth by carefully swirling.

For mTSB with novobiocin acc. USDA-FSIS (mTSB+n), final concentration of novobiocin sodium salt is 8 mg per liter.

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

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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 of *Escherichia coli* O157:H7, 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 (static) with their contents for 15 h to 24 h at 42 ± 1°C. After incubation is complete, follow the procedure as given by USDA-FSIS.

Following the procedure given by USDA-FSIS for outbreak-related samples of fermented sausage or cooked meat products for detection of *Escherichia coli* O157:H7, place each 25 ± 1 g sample or sub-sample in a sterile strainer bag and add 225 ± 4,5 ml of mTSB+n. Pummel, blend or hand massage until clumps are dispersed. Incubate all bags (static) with their contents for 15 h to 22 h at 42 ± 1°C. After incubation is complete, follow the procedure as given by USDA-FSIS.

Following the procedure given by USDA-FSIS for detection of *Salmonellae* from meat carcasses and environmental sponges, add the mTSB to the sample bag containing a sponge moistened with 10 ml of buffer to bring the total volume to 60 ml. Mix well. If the project calls for a larger volume of buffer, adjust the volume of enrichment broth to a 1:6 ratio. Incubate 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.

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Quality Control

Without addition of Novobiocin for *Salmonella* spp.:				
Function	**Control strains**	**Incubation**	**Method of control**	**Criteria and expected results**
Productivity	*Salmonella* Typhimurium ATCC® 14028 [WDCM 00031] + *Staphylococcus aureus* ATCC® 25923 [WDCM 00034]	15-21 hrs at 41-43 °C aerobic	Qualitative	> 10 red colonies on Rambach® agar
	Salmonella Enteritidis ATCC® 13076 [WDCM 00030] + *Staphylococcus aureus* ATCC® 25923 [WDCM 00034]			
	Salmonella Abaetetuba ATCC® 35640 + *Staphylococcus aureus* ATCC® 25923 [WDCM 00034]			
	Salmonella Cholerasuis ATCC® 13312 + *Staphylococcus aureus* ATCC® 25923 [WDCM 00034]			
With addition of Novobiocin for *Escherichia coli* O157:H7:				
Function	**Control strains**	**Incubation**	**Method of control**	**Criteria and expected results**
Productivity	*Escherichia coli* Serogroup O157:H7 ATCC® 35150 + *Staphylococcus aureus* ATCC® 25923 [WDCM 00034]	15-21 hrs at 41-43 °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]			
	Escherichia coli ATCC® 25922 [WDCM 00013] + *Staphylococcus aureus* ATCC® 25923 [WDCM 00034]			if growth: pink colonies on SMAC agar*

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Selectivity	*Staphylococcus aureus* ATCC® 25923 [WDCM 00034]	15-21 hrs at 41-43 °C aerobic	Qualitative	Total inhibition on TSA
Without addition of Novobiocin for non-O157 Shiga Toxin-producing *E. coli*:				
Function	**Control strains**	**Incubation**	**Method of control**	**Expected results**
Productivity	*Escherichia coli* Serogroup 026:H4 ATCC® BAA-2212	15-21 hrs at 41-43 °C aerobic	Qualitative	Growth good to very good
	Escherichia coli Serogroup 45:H10 ATCC® BAA-2649			
	Escherichia coli Serogroup 0103 ATCC® BAA-2214			
	Escherichia coli Serogroup 0111a,11b:K58:H21 ATCC® 29552			
	Escherichia coli Serogroup O121 ATCC® BAA-2190			
	Escherichia coli Serogroup 0145:H34 ATCC® BAA-2216			

* SMAC Agar: Sorbitol MacConkey Agar

Please refer to the actual batch related Certificate of Analysis.

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

Literature

ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. EN ISO 11133:2014+Amd1:2018.

USDA-FSIS United States Department of Agriculture – Food Safety and Inspection Service. Laboratory Guidebook Chapter MLG 4.09 (2017): Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, and 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 5.09 (2015): Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products and Carcass and Environmental Sponges. Washington D.C., USA.

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USDA-FSIS United States Department of Agriculture – Food Safety and Inspection Service. Laboratory Guidebook Chapter MLG 5B.05 (2014): Detection and Isolation of non-O157 Shiga Toxin-Producing *Escherichia* coli (STEC) from Meat 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 Appendix 1.09 (2017) 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
mTSB broth Selective Supplement (8 mg Novobiocin)	1.20593.0010	10 x 1 vial
GranuCult® prime mTSB broth with Novobiocin (20 mg/L) acc. ISO 16654	1.09205.0500	500 g
NutriSelect® prime Sorbitol-MacConkey Agar (SMAC Agar) acc. ISO 16654 and FDA-BAM	1.00213.0500	500 g
CT Supplement for microbiology	77981	5 x 1 vial
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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