Technical Data Sheet

GranuCult® prime
Hektoen enteric (HE) agar acc. ISO 21567, FDA-BAM and ref. to ISO 6579

Ordering number: 1.00215.0500

For the isolation and differentiation of *Salmonella* and *Shigella* from the food chain and other materials.

Hektoen enteric (HE) agar acc. ISO 21567, FDA-BAM and ref. to ISO 6579 is also known as HEA and HE agar. It has been named after the Hektoen Institute for Medical Research, Chicago, Illinois, USA.

This culture medium complies with the specifications given by EN ISO 21567, FDA-BAM Medium M61, APHA and reference to 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

This culture medium provides good colonial differentiation and inhibits some coliforms and other non-lactose-fermenting bacteria, thereby facilitating the isolation and differentiation of *Salmonella* spp. and *Shigella* spp. from samples from the food chain and other materials. Lactose-positive colonies have a clearly different color from lactose-negative colonies due to the presence of the two indicators for carbohydrate dissimilation: bromothymol blue and acidic fuchsin. Due to the presence of two additional carbohydrates - sucrose (saccharose) and salicin - this color difference is also observed for colonies, which can only slowly ferment lactose. The combination of thiosulfate as a reactive compound with an iron salt as an indicator causes H_2S-positive colonies to become black in color.

This culture medium is moderately to highly selective, the mixture of bile salts suppresses the growth of most of the accompanying microorganisms. The inhibitory effect of bile salts on the growth of *Shigella* spp. is reduced by relatively large amounts of peptone and carbohydrates which allow good growth. Agar is the solidifying agent.

Typical Composition

EN ISO 6579-1 specifies no composition for Hektoen Enteric agar.

Specified by EN ISO 21567		Specified by FDA-BAM Medium M61		GranuCult® prime Hektoen enteric (HE) agar acc. ISO 21567, FDA-BAM and ref. to ISO 6579	
Enzymatic digest of meat	12.0 g/l	Peptone	12 g/l	Enzymatic digest of meat	12.0 g/l
NaCl	5.0 g/l	NaCl	5 g/l	NaCl	5.0 g/l
Yeast extract	3.0 g/l	Yeast extract	3 g/l	Yeast extract	3.0 g/l
Saccharose	12.0 g/l	Sucrose	12 g/l	Saccharose*	12.0 g/l
Lactose	12.0 g/l	Lactose	12 g/l	Lactose	12.0 g/l
Salicin	2.0 g/l	Salicin	2 g/l	Salicin	2.0 g/l
Sodium thiosulfate	5.0 g/l	Sodium thiosulfate	5 g/l	Sodium thiosulfate	5.0 g/l
Ammonium ferric citrate	1.5 g/l	Ferric ammonium citrate	1.5 g/l	Ammonium iron(III) citrate **	1.5 g/l
Bile salts No. 3	9.0 g/l	Bile salts No. 3	9 g/l	Bile salts No. 3	9.0 g/l
Bromothymol blue	0.065 g/l	Bromothymol blue	0.065 g/l	Bromothymol blue	0.065 g/l
Acid fuchsin	0.1 g/l	Acid fuchsin	0.1 g/l	Fuchsin acid	0.1 g/l
Agar	12 g to 18 g/l***	Agar	14 g/l	Agar-agar****	13.5 g/l
Water	1000ml	Water	1 liter	Water	n/a
pH at 25 °C	7.5 ± 0.2	pH at 25 °C	7.5 ± 0.2	pH at 25 °C	7.5 ± 0.2

* Saccharose is synonymous with Sucrose.
**Ammonium iron(III) citrate is synonymous with Ammonium ferric citrate and with Ferric ammonium citrate.
*** Depending on the gel strength of the agar.
****Agar-Agar is equivalent to other different terms of agar.

Preparation

Dissolve 75.0 g in 1 liter of purified water. Heat gently in boiling water and agitate frequently until completely dissolved. Do not autoclave!

The dehydrated medium is a granulate with dark-green color.
The prepared medium is clear to slightly opalescent and blue-green to blue-grey. The pH value at 25 °C is in the range of 7.3 - 7.7.
Before inoculation, allow the prepared medium to equilibrate at room temperature if it was stored at a lower temperature.
There should be no visible moisture on the plates before use. When moisture is present, the plates should be dried for the minimum time required to remove visible moisture, following the procedure as described by EN ISO 11133.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Following the procedure given by EN ISO 6579-1, EN ISO 21567, FDA-BAM and APHA, inoculate the surface of the medium from

the selective enriched cultures so that well-isolated colonies will be obtained.

Incubate the inoculated plates inverted under aerobic conditions, e.g.

- acc. to EN ISO 6579-1 at (37 ± 1 °C) for (24 ± 2 h);
- acc. to EN ISO 21567 at (37 ± 1 °C) for between 20 h and 24 h;
- acc. to FDA-BAM Chapter No. 5 at (35 ± 2 °C) for (24 ± 2 h);
- acc. to APHA Chapter No. 37 at (35 ± 2 °C) for (16 to 24 h).

On Hektoen enteric (HE) agar acc. ISO 21567, FDA-BAM and ref. to ISO 6579, *Salmonella* spp. produce transparent green to blue-green colonies, with or without black centres, or may appear as almost completely black colonies due to H_2S production.
Shigella spp. produce green, transparent and moist colonies.
Lactose-, sucrose- or salicin-fermenting Gram-negative bacteria may be inhibited or may produce red to salmon colored colonies with or without precipitation zone.

Colonies of the most important bacteria usually have the appearance described below:

Appearance of colonies	**Microorganisms**
Green, moist, transparent	*Shigella, Providencia*
Green to blue-green, with or without a black centre	*Salmonella, Proteus*
Green to bluish, flat, irregular edge	*Pseudomonas*
Red to salmon surrounded by a zone of precipitate	Coliform bacteria

This presumptive evidence must be confirmed by carrying out the usual 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.

Self-prepared plates can be stored in the dark and protected against evaporation:

- acc. to ISO 21567 at (5 ± 3 °C) for up to 2 weeks;
- acc. to FDA-BAM at (4 ± 2 °C) for up to 30 days;
- acc to Corry et al. (2012) at (5 ± 3 °C) for up to 3 weeks.

Microbiological Performance

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

Test method: Performance testing of solid culture media - Qualitative testing

Test strain	Specification	
	Growth	Typical reaction
Salmonella Typhimurium ATCC® 14028 [WDCM 00031]	good	blue-green colonies with black centre, without precipitation zone
Salmonella Enteritidis ATCC® 13076 [WDCM 00030]	good	blue-green colonies with black centre, without precipitation zone
Shigella flexneri ATCC® 12022 [WDCM 00126]	good	green and moist colonies, without black centre, without precipitation zone
Shigella flexneri ATCC® 29903 [WDCM 00125]	good	green and moist colonies, without black centre, without precipitation zone
Shigella sonnei ATCC® 29930 [WDCM 00127]	good	green and moist colonies, without black centre, without precipitation zone
Proteus mirabilis ATCC® 29906 [WDCM 00023]	weak to good	green to blue-green colonies, with or without black centre, without precipitation zone
Escherichia coli ATCC® 25922 [WDCM 00013]	weak to good	red to salmon colonies, without black centre, with precipitation zone
Enterococcus faecalis ATCC® 29212 [WDCM 00087]	none to weak	If growth: salmon or yellowish colonies, without black centre, without precipitation zone

Incubation: 22 ± 2 h at 37 ± 1 °C, aerobic.

Please refer to the actual batch related Certificate of Analysis.

Literature

APHA (2015) Chapter No. 37: *Shigella*. and Chapter No. 67: Microbiological media, reagents and stains. Compendium of Methods for the Microbiological Examination of Foods. 5th ed. American Public Health Association, Washington, D.C.

EN ISO International Standardisation Organisation. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* - Part 1: Horizontal method for the detection of *Salmonella* spp. + Amendment 1. EN ISO 6579-1:2017/Amd1:2020.

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/Amd1:2018/Amd2:2020.

EN ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Shigella* spp. EN ISO 21567:2004.

FDA-BAM (2020): Chapter No. 5: *Salmonella*. U.S. Food and Drug Administration - Bacteriological Analytical Manual.

FDA-BAM (2018): Media Index for BAM - BAM Media M61: Hektoen Enteric (HE) Agar. Food and Drug Administration - Bacteriological Analytical Manual.

Andrews, W.H., Wilson, C.R., Poelma, P.L. and Romero, A. (1979): Relative Productivity of Five Selective Plating Agars for the Recovery of *Salmonella* from Selected Food Types. J. Assoc. Off. Anal. Chem. **62**: 320-326.

Andrews, W.H., Poelma, P.L. and Wilson, C.R. (1981): Comparative Efficiency of Brilliant Green, Bismuth Sulfite, Salmonella-Shigella, Hektoen Enteric, and Xylose Lysine Desoxycholate Agars for the Recovery of *Salmonella* from Foods: Collaborative Study. J. Assoc. Off. Anal. Chem. **64**: 899-928.

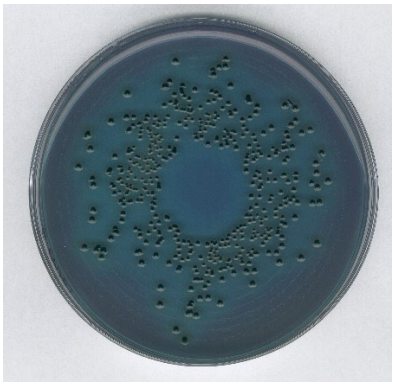
Bisciello, N.B. jr. and Schrade, J. (1974): Evaluation of Hektoen Enteric Agar for the detection of *Salmonella* in foods and feeds. J. Assoc. Off. Anal. Chem. **57**: 992-996.

Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. (2012): Hektoen Enteric (HE) agar. In: Handbook of Culture Media for Food and Water Microbiology, pp. 772-774. Royal Society of Chemistry, Cambridge, UK.

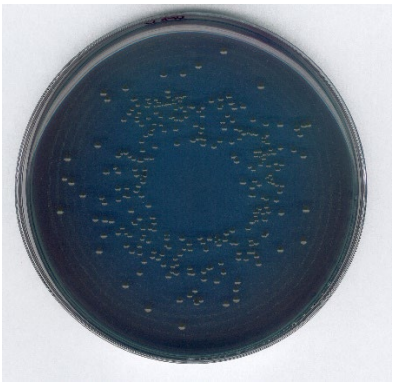
Islam, M.A. (2012): Culture media for the isolation of *Shigella* spp. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds). pp. 287-297. Royal Society of Chemistry, Cambridge, UK.

King, S. and Metzger, W.J. (1968): A new plating medium for the isolation of enteric pathogens. I. Hektoen Enteric Agar. Appl. Microbiol. **16**: 557-578.

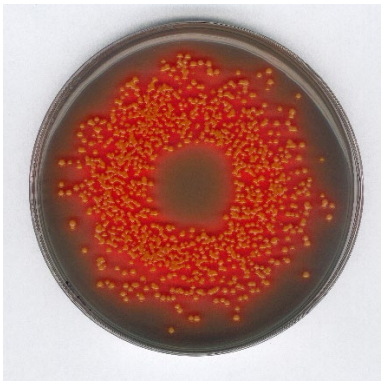
Mooijman, K.A. (2012): Culture media for the isolation of *Salmonella*. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird,R.M. eds). pp. 261-286. Royal Society of Chemistry, Cambridge, UK.



Salmonella Enteritidis
ATCC® 13076
[WDCM 00030]



Shigella flexneri
ATCC® 12022
[WDCM 00126]



Enterobacter cloacae
ATCC® 13047
[WDCM 00083]

Ordering Information

Product	Cat. No.	Pack size	Other pack sizes available
GranuCult® prime Hektoen enteric (HE) agar acc. ISO 21567, FDA-BAM and ref. to ISO 6579	1.00215.0500	500 g	
GranuCult® Buffered Peptone Water acc. ISO 6579, ISO 21528, ISO 22964, FDA-BAM and EP	1.07228.0500	500 g	5 kg, 10 kg, 25 kg
GranuCult® MKTTn (MULLER-KAUFFMANN Tetrathionate Novobiocin) Broth (Base) acc. ISO 6579	1.05878.0500	500 g	
GranuCult® RVS (RAPPAPORT-VASSILIADIS-Soya) Broth (Base) acc. ISO 6579	1.07700.0500	500 g	
Novobiocin Sodium Salt	N6160-1-G	1 g	
MSRV (Modified Semi-solid RAPPAPORT - VASSILIADIS) Medium (Base) acc. ISO 6579	1.09878.0500	500 g	
MSRV Selective Supplement	1.09874.0010	1 x 10 vials	

ChromoCult® RAMBACH® agar ref. to ISO 6579 (Kit)	1.00188.0002	4 x 250 ml	
ChromoCult® RAMBACH® agar ref. to ISO 6579 (Kit)	1.00188.0004	4 x 1000 ml	
GranuCult® prime Bismuth sulfite (BS) agar acc. WILSON and BLAIR acc. FDA-BAM	1.00191.0500	500 g	
GranuCult® prime BPLS (Brilliant-green Phenol-red Lactose Sucrose) agar ref. to ISO 6579	1.00207.0500	500 g	
Triple Sugar Iron Agar	1.03915.0500	500 g	
Urea Agar (Base) acc CHRISTIANSEN acc. ISO 6579, ISO 10273, ISO 19250, ISO 21567	1.08492.0500	500 g	
Urea GR for Analysis ACS, Reagent Ph Eur	1.08487.0500	500 g	
MR-VP (Methyl Red-VOGES-PROSKAUER) Broth	1.05712.0500	500 g	
KOVACS' Indole Reagent	1.09293.0100	100 ml	