Technical Data Sheet

Chromocult®
RAMBACH® agar ref. to ISO 6579 (Kit)

Ordering number: 1.00188.0002 / 1.00188.0004

For the isolation and differentiation of *Salmonella* from food and animal feed, water and other materials.

RAMBACH® agar ref. to ISO 6579 is also known as Propylene glycol deoxycholate neutral red agar.

This culture medium complies with the specifications 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

RAMBACH® agar was one of the first chromogenic media containing a chromogenic substrate mix. After splitting the substrate as a result of a specific enzymatic activity, the chromophore is released and a coloured end product becomes visible and facilitates the identification. This culture medium uses acid formation from propylene glycol by *Salmonella* spp. which results in pink-reddish to crimson colonies. Additionally, β-D-galactosidase production by other members of the family *Enterobacteriaceae* is detected by reaction with the chromogenic substrate X-Gal (5-bromo-4-chloro-3-indoyl- β-D-galactopyranoside), which results in formation of blue-green colonies.

The combination of colors resulting from the two reactions differentiates most *Salmonella* spp. from competing organisms. *Salmonella* spp. which degrade propylene glycol to an acid, grow by the addition of a pH-indicator as characteristic pink-reddish to crimson colonies. *Salmonella* Typhi, *S*. Paratyphi A and B fail to produce acid from propylene glycol, resulting in colourless-yellow colonies.

Due to the cleavage of the chromogenic substrate, the galactosidase-positive *Enterobacteriaceae* grow as blue-green colonies. However, the strains of *Salmonella enterica* subspecies *arizonae* (IIIa) and *diarizonae* (IIIb) and of *Salmonella bongori* (V) produce β-D-galactosidase and therefore can also appear as blue-green or blue to purple-violet colonies. These particular subspecies may well be present in samples from sheep, turkeys, and cold-blood animals.

This culture medium enables *Enterobacteriaceae* to multiply readily by its nutritive substrate. Sodium deoxycholate inhibits the accompanying Gram-positive bacteria. Agar is the solidifying agent.

Typical Composition

EN ISO 6579-1 specifies no composition for Rambach® agar.

Chromocult® RAMBACH® agar ref. to ISO 6579 (Kit)	
Peptone	8.0 g/l
NaCl	5.0 g/l
Sodium deoxycholate	1.0 g/l
Chromogenic Mix	1.5 g/l
Propylene glycol	10.5 g/l
Agar-Agar*	15.0 g/l
Water	n/a
pH at 25 °C	7.3 ± 0.2

*Agar-Agar is equivalent to other different terms of agar.

Preparation

Add 1 vial of liquid-mix to distilled water and mix by swirling until completely dissolved.
(The water quantity is dependent on the respective pack-size.)
Add 1 vial of nutrient-powder and mix by swirling until completely suspended.
Heat in a boiling water-bath or in a current of steam, while carefully shaking from time to time.
The medium is completely dissolved, if no visual particles stick to the glass-wall.
The medium should not be heat-treated further!
Standard time for complete dissolution (shaking in 5 minutes sequence):
250 ml: 20–25 minutes; 1000 ml: 35–40 minutes.

Do not autoclave, do not overheat!

Cool the medium as fast as possible in a water-bath (45–50 °C).
During this procedure (max.: 30 minutes) gently shake the medium from time to time.
Pour into plates. To prevent any precipitate or clotting of the chromogenic mix in the plates, we advise to place Petri dishes – during the pouring procedure – on a cool (max. 25 °C) surface.

The dehydrated medium is a powder with dark-green color. The color of the supplement may vary. This does not negatively influence the function of the supplement or the culture medium.

The prepared medium is opaque and light pink. The pH value at 25 °C is in the range of 7.1 - 7.5.

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, 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 between 34 °C and 38 °C for (24 ± 3 h).

On RAMBACH® agar ref. to ISO 6579, *Salmonella* spp. which degrade propylene glycol to an acid, grow by the addition of a pH-indicator as characteristic pink-reddish to crimson colonies.

Galactosidase-positive *Enterobacteriaceae* grow as blue-green colonies. This includes also galactosidase-positive *Salmonella* spp. which form blue-green or blue to purple-violet colonies.

Proteus and *Pseudomonas* spp. grow as colorless to yellowish-orange colonies, sometimes with a soft pink center. *Pseudomonas* spp. shows a yellowish-green to blue coloration of the medium in areas of heavy growth.

Salmonella spp. which are not able to produce acid from propylene glycol, e.g. *Salmonella* Typhi, *S.* Paratyphi A and B, grow as colorless to yellowish colonies.

Gram-positive bacteria are largely inhibited by the addition of sodium deoxycholate.

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 Corry et al. (2012) at (5 ± 3 °C) for up to 14 days.

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	pink-reddish to crimson colonies
Salmonella Enteritidis ATCC® 13076 [WDCM 00030]	good	pink-reddish to crimson colonies
Salmonella Abaetetuba ATCC® 35640 [WDCM -]	good	pink-reddish to crimson colonies
Salmonella Abortivoequina ATCC® 9842 [WDCM -]	good	colourless to yellowish colonies
Salmonella Arizonae ATCC® 13314 [WDCM -]	good	blue to purple-violet colonies
Salmonella Diarizonae ATCC® 12325 [WDCM -]	good	purple-violet colonies
Escherichia coli ATCC® 25922 [WDCM 00013]	weak to good	bluish-greenish colonies
Klebsiella pneumoniae ATCC® 13883 [WDCM 00097]	weak to good	blue to blue violet colonies
Proteus mirabilis ATCC® 29906 [WDCM 00023]	weak to good	colourless to yellowish colonies
Pseudomonas aeruginosa ATCC® 27853 [WDCM 00025]	no limit	colourless to yellowish-orange colonies; yellowish-green to blue colouration of the medium in areas of heavy growth
Staphylococcus aureus ATCC® 25923 [WDCM 00034]	none	n.a.
Bacillus cereus ATCC® 11778 [WDCM 00001]	none	n.a.

Incubation: 22 ± 2 h at 35 ± 1 °C, aerobic.

Please refer to the actual batch related Certificate of Analysis.

Literature

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.

Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. (2012): Rambach agar (Propylene glycol deoxycholate neutral red agar). In: Handbook of Culture Media for Food and Water Microbiology, pp. 898-900. Royal Society of Chemistry, Cambridge, UK.

Gruenewald, R., Henderson, R.W. and Yappow, S. (1991): Use of Rambach Propylene Glycol conatining agar for identification of *Salmonella* spp. J. Clin. Microbiol. **29**: 2354-2356.

Joosten, H.M.L.J., van Dick, W.G.F.M and van der Velde, F. (1994): Evaluation of motility enrichment on modified semisolid Rappaport-Vassiliadis medium (MSRV) and automated conductance in combination with Rambach agar for *Salmonella* detection in environmental samples of a milk powder factory. Uint. J. Food Microbiol. **22**: 201-206.

Kühn, H., Wonde, B., Rabsch, W. and Reissbrodt, R. (1994): Evaluation of Rambach agar for detection of *Salmonella* subspecies I to IV. Appl. Env. Microbiol. **60**: 749-751.

Monfort, P., Le Gal, D., Le Saux, J.C., Piclet, G., Raguenes, P., Boulben, S. and Plusquellec, A. (1994): Improved rapid method for isolation and enumeration of salmonella from bivalves using Rambach agar. J. Microbiol. Meth. **19**: 67-79.

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.

Rambach, A. (1990): New plate medium for facilitated differentiation of *Salmonella* spp. from *Proteus* spp. and other enteric bacteria. Appl. Env. Microbiol. **56**: 301-303.

El-Sherif, A.M. and Elmossalami, M.K. (1998): Rambach agar as a new plate differential medium for the identification of some enteric pathogens in meat products. Z. Lebensm. Unters. Forsch. **207**: 160–163.

Salmonella Enteritidis
ATCC® 13076
[WDCM 00030]

Escherichia coli
ATCC® 25922
[WDCM 00013]

Proteus mirabilis
ATCC® 29906
[WDCM 00023]

Red boat: *Salmonella* Typhimurium
Green sails: *Escherichia coli*
Blue waves and birds: *Klebsiella pneumoniae*
Colourless sun: *Shigella flexneri*

Ordering Information

Product	Cat. No.	Pack size
Chromocult® RAMBACH® agar ref. to ISO 6579 (Kit)	1.00188.0002	Kit for 4x 250 ml
Chromocult® RAMBACH® agar ref. to ISO 6579 (Kit)	1.00188.0004	Kit for 4x 1000 ml