Dr. Möller & Schmelz GmbH
Corporation for Applied Microbiology

Nutrient media for the microbiological quality control in breweries

Content

Beer – a drink with character for untarnished enjoyment

Beer always was a topic for extensive discussions, preferably with a freshly tapped beer in your hand. Since the beginning of the art of brewing it is all about taste, ingredients, enjoyment – and about quality assurance by the master brewer.

There are a lot of regulations, most of them are regional, that guarantee what beer is allowed to contain. The most popular is probably the Bavarian “Reinheitsgebot” (purity requirements) from 23. April 1516.

Malt, hops, yeast and water are the essential components that are used in brewing and lead to a wide variety of flavours.

Master brewers know: Not only flavour is crucial. Also the declared harmlessness of the beer for the connoisseur is also of decisive importance for market success – as it is for other beverages, too.

The quality of the raw materials used, the purity of the yeast cultures and the controlling of the entire brewing process up to bottling are important factors.

Wild yeasts and bacterial contaminations may lead to off-flavours and to spoilage of the beer and thus cause financial damage and even loss of image.

Culture media for the microbiological quality control and monitoring by **Dr. Möller & Schmelz** supports those responsible in breweries and the beverage industry in their daily routine.

We are there for you personally if you have any questions or suggestions. Please do not hesitate to contact us.

Cheers!

Graduate Biologist Michael Sawatzki
General Manager Dr. Möller & Schmelz GmbH


CERTIFICAT
DIN EN ISO 9001:2015
DIN EN ISO 14001:2015

AQM GmbH hereby confirms the standard conformity of the DIN EN ISO 9001: 2015 and 14001: 2015, for the company

M&S Dr. Möller & Schmelz GmbH
Gesellschaft für angewandte Mikrobiologie
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37079 Göttingen

The scope of application is valid for the production and distribution of nutrient pad set, nutrient media and filtration equipment.

The company has proved in a conformity assessment according to DIN EN ISO / IEC 17050-1, Report no. 2019-12-21, demonstrated that its quality and environmental management meets the requirements of the standards

DIN EN ISO 9001:2015
DIN EN ISO 14001:2015

and is applied in the company.

Certificate Registration No.: 2002.1034
This certificate is valid until: 31.12.2022
The company has been certified since: 2001
Acheq: 18.12.2019

AQM

Sarah Brodhel
DQG/DQG Quality Auditor

Components and process steps of beer production

The characteristics and quality of a beer are influenced by many factors.

In the following we only consider the microbiological aspects that affect both the taste and the safety of the beverage. For a better overview, we have chosen a subdivision into components and different brewing process steps.

Brewing water

There is a saying among brewers: *"Brewing water always is drinking water, but drinking water isn't always brewing water"*. Water is the main component of beer with more than 90% and therefore has a very great influence on the beverage. For this reason, it is important to monitor the quality of this component, no matter, whether water from your own well or municipal water is used.

The requirements for drinking water are set in the EU-directive 98/83 on the quality of water intended for human consumption and are regulated by ordinances in the individual member states. It is specified in the microbiological section:

- *Escherichia coli* / Coliforms: 0 cfu in 100 ml (ISO 9308-1:2017-09)
- Enterococci: 0 cfu in 100 ml (ISO 7899-2:2000)
- *Pseudomonas aeruginosa*: 0 cfu in 100 ml (16266:2006)
- Total heterotrophic count at 22°C: 100 cfu in 1 ml (ISO 6222:1999)
- Total heterotrophic count at 36°C: 20 cfu in 1 ml (ISO 6222:1999)
- *Clostridium perfringens*: 0 cfu in 100 ml

For the monitoring of the water in the entire brewing process following parameters are recommended:

Determination of total heterotrophic count from 1 ml water by **pour plate method** using **Nutrient-Agar *items 4080 or 5080*** or **Standard-Agar *items 4135 or 5101***, incubation 3 days aerobic at 22°C and 1 day aerobic at 36°C.

Determination of Coliforms from 100 ml water by **membrane filtration method** using **Colichrom-Agar *items 4028 or 5025*** or **Colichrom-NPS *item 1035***, incubation 24 hours aerobic at 36°C.

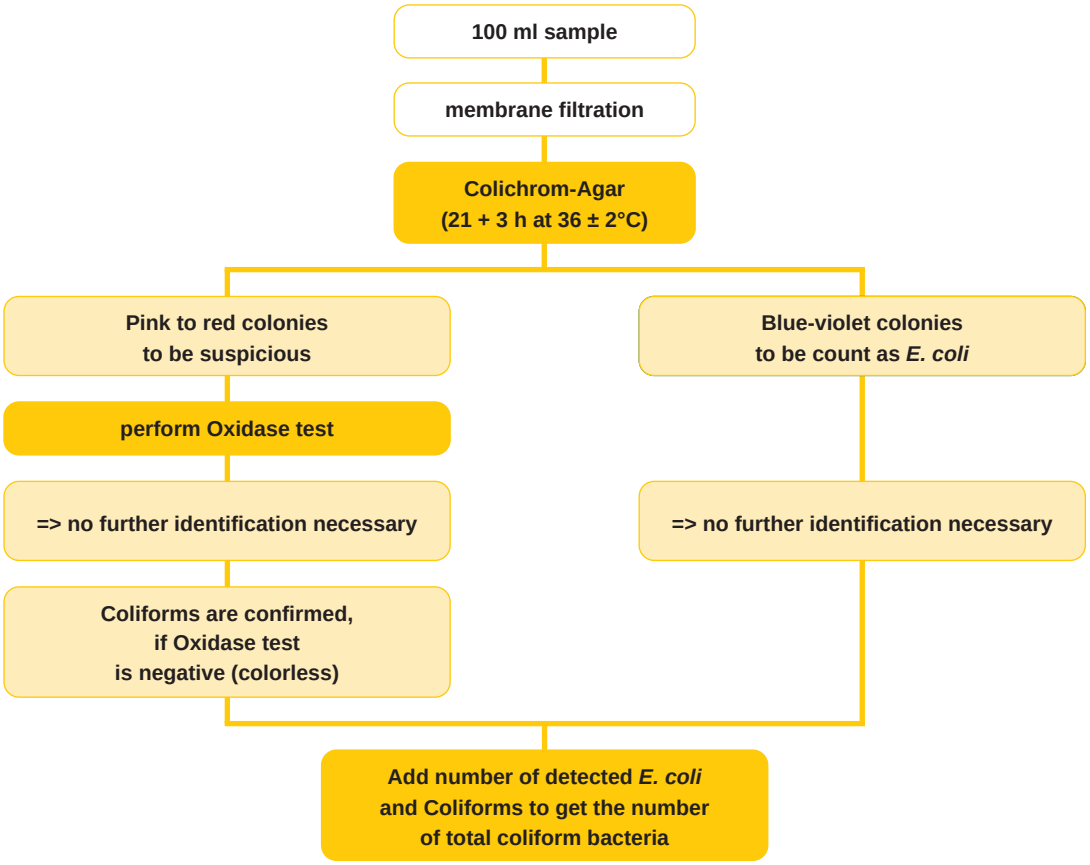
Determination of yeasts and Acetobacteraceae from 100 ml water by **membrane filtration method** using **Wort-Agar *items 4150 or 5110*** or **Wort-NPS *item 1260***, incubation 2-3 days aerobic at 25°C.

Determination of lactic acid bacteria and beer-spoiling microorganisms from 100 ml water by **membrane filtration method** using **MRS-Agar *items 4061 or 5061*** or **MRS-NPS *item 1110*** or **Beer-Agar *item 5015*** or **Beer-NPS *item 1020***, incubation 2-4 days microaerophilic at 30°C.

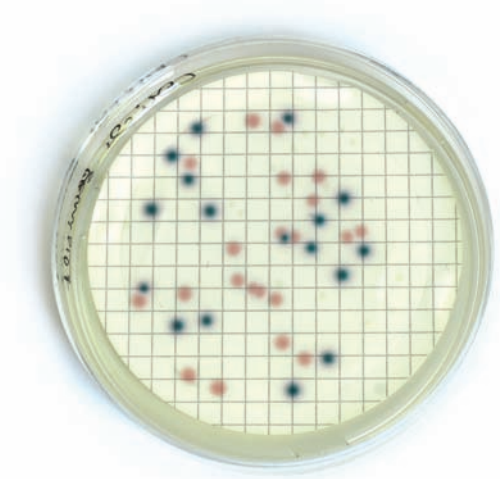
One possibility to quickly determine the number of *E. coli* and Coliforms is using the selective chromogenic **Colichrom-Agar** according to ISO 9308-1:2017-09.

The chromogenic substances contained allow easy identification of *E. coli*, which appear as blue-violet colonies, whereas Coliforms are pink to red.
Gram-negative bacteria other than coliform bacteria grow in beige colors.

The advantage of this medium is that *E. coli* and coliform bacteria can be detected simultaneously on the same medium within 24 hours.



Test scheme with M&S Colichrom-Agar, *items 4028 or 5025*



Mixed culture of *E. coli* WDCM00012 (blue) and *Enterobacter aerogenes* (pink) WDCM00175 on Colichrom-Agar, incubated for 24 hours at 36°C

Pour plate method

1. Melt the agar in a boiling water bath until it is completely liquefied. Cool down to 48-50°C.
   Note: Make sure that the cap is loosened in order to allow pressure equalization during this process.
2. Pipet 1 ml of the sample into a sterile Petri dish. After addition of 10 ml (60 mm Petri dish) or 15-20 ml (90 mm Petri dish) of the tempered agar gently mix both components.
3. After solidification incubate the Petri dish with the lid facing down.
   Incubation conditions (temperature and time) are dependent on the agar medium and the target organisms.
4. The number of colonies is determined by counting the colonies visible with a 6-8 fold magnification and is defined per ml.
   Note: Growth and positive results with selective media are to be considered as indication only. For safe diagnosis further tests are necessary.

Membrane filtration method

1. Melt the agar in a boiling water bath until it is completely liquefied. Cool down to 48-50°C.
   Note: Make sure that the cap is loosened in order to allow pressure equalization during this process.
2. For preparation of the agar plates the cooled agar is poured into sterile Petri dishes.
   About 10 ml is needed for a 60 mm Petri dish, 15-20 ml are required for a 90 mm Petri dish.
   Allow the agar to solidify.
3. For filtration of the sample through a suitable membrane filter follow the manufacturer's instruction for use of the filtration system.
4. After filtration remove membrane filter from the frit with a sterile tweezers and place it on the prepared nutrient agar without catching air bubbles.
5. Incubate the Petri dish with the lid facing down. Incubation conditions (temperature and time) are dependent on the agar medium and the target organisms.
   Note: Growth and positive results with selective media are to be considered as indication only. For safe diagnosis further tests are necessary.

Nutrient pad sets (NPS) are sterile dehydrated nutrient culture media that are ready for immediate use after addition of sterile water. They are made of biologically inert cellulose cardboard that serves as a substrate for the nutrient solution. Since it does not bind the nutrients either chemically or physically, the nutrient components are completely available for the growth of microorganisms.

The composition of the various nutrient solutions used for the various NPS comply with the formulations specified in the relevant standards and regulations.

Nutrient pad sets have a number of advantages over conventional agar media:

- Storage at room temperature
- Shelf life of up to 2 years
- Ready for immediate use after moistening
- Easy handling

Instruction for using NPS

As a general point, make sure that all devices are sterile and that the rules for sterile work are followed.

1. Open a pack of ten and remove a Petri dish containing a nutrient pad.
2. Add 3-3.5 ml sterile, distilled or demineralized water to the nutrient pad in the Petridish.
   Moisture level is optimal, if an excess ring of liquid is clearly visible.
3. Open sealed envelope, remove membrane filter with sterile tweezers, place the membrane filter on top of the frit of the filter holder and put on the filter funnel.
4. Filter sample. Rinse with sterile water or peptone water and remove excess liquid carefully from the filter by extended vacuum.
   Note: For using the filtration device please follow the manufacturer's instruction.
5. Carefully remove the membrane filter from the frit with a sterile tweezers and place it on the prepared nutrient pad (pls. see above) without catching air bubbles. Incubate the Petri dish with the lid facing upwards. The incubation conditions are dependent on the NPS-grade and the target microorganisms.
   Note: Growth and positive results with selective media are to be considered as indication only. For safe diagnosis further tests are necessary.

For an overview about our NPS product line see
www.moeller-schmelz.de/nps-orderinginformation.html

Overview about media for brewing water analysis

Item	Item No	Recommendation for incubation
Beer-Agar	5015 (4 x 250 ml)	2-4 days at 30°C
Beer-NPS	1020 (50 tests) 1020-H (100 tests)	2-4 days at 30°C
Colichrom-Agar	4028 (25 x 20 ml) 4028-100 (100 x 20 ml) 5025 (4 x 250 ml) 5025-24 (24 x 250 ml)	24 h at 36°C
Colichrom-NPS	1035 (50 tests) 1035-H (100 tests)	24 h at 36°C
MRS-Agar	4061 (25 x 20 ml) 4061-100 (100 x 20 ml) 5061 (4 x 250 ml) 5061-24 (24 x 250 ml)	2-4 days at 28°C
MRS-NPS	1110 (50 tests) 1110-H (100 tests)	2-4 days at 28°C
Nutrient-Agar	4080 (25 x 20 ml) 4080-100 (100 x 20 ml) 5080 (4 x 250 ml) 5080-24 (24 x 250 ml)	3 days at 22°C and 1 day at 36°C
Standard-Agar	4135 (25 x 20 ml) 4135-100 (100 x 20 ml) 5101 (4 x 250 ml) 5101-24 (24 x 250 ml)	3 days at 22°C and 1 day at 36°C
Wort-Agar	4150 (25 x 20 ml) 5110 (4 x 250 ml) 5110-24 (24 x 250 ml)	2-3 days at 25°C
Wort-NPS	1260 (50 tests) 1260-H (100 tests)	2-3 days at 25°C

Detailed technical data sheets are available at
www.moeller-schmelz.de


DR. MÖLLER & SCHMELZ GmbH
Gesellschaft für angewandte Mikrobiologie

Certificate of Quality

Nutrient media from Dr. Möller & Schmelz are manufactured of high-quality raw materials from certified suppliers and produced and tested with respect to product identity, sterility, pH-value, performance, growth and function in accordance with the requirements of the GMP-code and ISO 11133.

Nutrient-Agar

Order No.	4080
No. of units	25 x 20ml
Lot No.	4504080A
Sterilization	2020-11
Expiry date	2021-06
storege temp.	4 - 12 °C

I confirm that the delivered lot meets all requirements of our strict quality assurance program.

Katrin Kühn
Qualitätssicherung

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CERTIFICATE

Yeasts and Wort

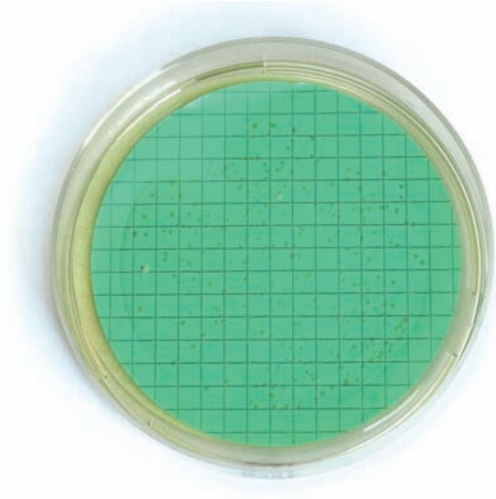
The purity of the pitching yeast is of particular importance for perfect beer quality. It may not contain any beer-spoiling microorganisms like Pediococci, Lactic bacteria or wild yeasts in order to avoid spoilage and off-flavours.

Testing for beer-spoiling bacteria and lactic acid bacteria by means of

- Inoculation of **BfB-Broth for beer-spoiling microorganisms** with indicator ***item 4008*** with 1 ml of sample.
- Plating of 0.1 ml of sample on **Beer-Agar *item 5015.***
- **Membrane filtration method** of 100 ml of sample on **Beer-Agar *item 5015*** or **Beer-NPS *item 1020.*** Incubation 2-4 days microaerophilic at 30°C.

The most common beer spoilers like Pediococcus, Pectinatus and other Lactobacteria can be enriched with the BfB-Broth for beer-spoiling microorganisms with indicator developed by **Dr. Möller & Schmelz**. Evidence is provided by clouding and colour change of the medium from reddish-brown to yellow.

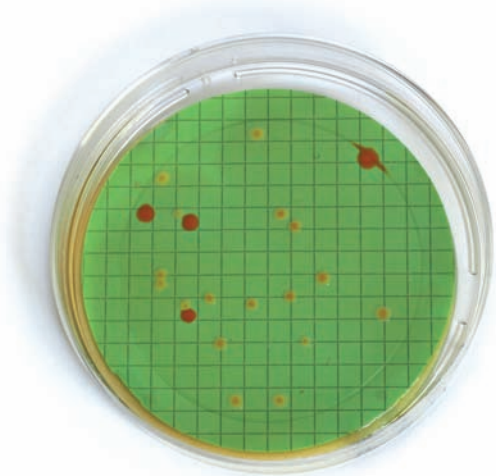
BfB-Broth for beer-spoiling microorganisms with indicator – each inoculated with 1 µl of an overnight culture and incubated microaerophilic for 72 hours at 30°C – from the left:
1. negative control 2. *Pediococcus damnosus* WDCM 00022
3. *Leuconostoc pseudomesenteroides* DSM 20193 4. *Pectinatus sp.* wildtype strain



Beer-NPS
Mixed culture of *Lactobacillus plantarum* DSM 20205 and *Pediococcus pentosaceus* WDCM 00158 – Incubation for 72 hours microaerophilic at 30°C

Testing for (Saccharomyces-) Wild Yeasts by means of

- Inoculation of **BfW-Broth for Wildtype Yeasts** with indicator ***item 4056*** with 1 ml of sample.
- Plating of 0.1 ml of sample on **Lysine-Agar *items 4055 or 5042*** or on **Copper Sulphate-Agar *item 5039*** or on **Crystal Violet-Agar *items 4051 or 5038*** incubation 2-5 days aerobic at 25°C.
- **Membrane filtration method** of 100 ml of sample on **Lysine-Agar *items 4055 or 5042*** or on **Lysine-NPS *item 1095*** or on **Copper Sulphate-Agar *item 5039*** or on **Crystal Violet-Agar *items 4051 or 5038*** incubation 2-5 days aerobic at 25°C.
- **Membrane filtration method** of 100 ml of sample on **Brettanomyces-NPS *item 1025*** incubation 4-7 days aerobic at 25°C.



Brettanomyces-NPS
Mixed culture of *Brettanomyces bruxellensis* DSM 70001 (yellow) and *Dekkera bruxellensis* Wild strain (orange) – incubation 6 days aerobic at 25°C

Different Wild Yeasts can be enriched with the BfW-Broth for Wildtype Yeasts with indicator item 4056 developed by **Dr. Möller & Schmelz**. Evidence is provided by clouding and colour change of the medium from reddish-brown to yellow. The growth of culture yeasts is widely inhibited.

BfW-Broth for Wildtype Yeasts with indicator
For the realistic implementation of the analysis,
1 ml of hopped beer was first added to the broth
and only then was inoculated with the yeast strains.
The incubation was carried out aerobically for 6 days at 25°C
***(B. bruxellensis)* and 2 days at 25°C (Wildtype strain) – from the left:**
1. negative control 2. *Brettanomyces bruxellensis* DSM 70001
3. Wildtype yeast from young wine

This is what our customer Familien-brauerei M. Ketterer GmbH & Co. KG says:

„The Dr. Möller & Schmelz GmbH has long been one of our valued suppliers of microbiological culture media. We feel very confident by the product quality and reliability to agreed delivery dates as well as the simple and direct communication. The trusting cooperation supports us in ensuring the high quality standards that we place on our drinks.“

Detection of *Saccharomyces diastaticus*

Inoculation of **Diastaticus-Broth with Durham tube *item 4029*** with 1 ml of sample.
Incubation aerobically/anaerobically for 3-5 days at 25°C.

S. diastaticus is able to ferment starch. This is shown by turbidity, colour change of the medium from blue to green and formation of gas, which is collected in the Durham tube.

Culture yeasts do not grow in this medium.

Diastaticus-Broth with Durham tube
For the realistic performance of the analysis, 1 ml of hopped beer was first added to the broth and only then was inoculated with the yeast strains.
The incubation was carried out aerobically for 4 days at 25°C – from the left:
1. negative control 2. *Saccharomyces diastaticus* DSM 70487
3. *Saccharomyces diastaticus* wild type strain from contaminated beer

Evaluation of fermentation performance

The fermentation power of yeasts can be rated by enrichment in
Malt-Broth with Durham tube *item 4058*.
This is especially of interest, if you want to check whether the vitality of the yeast used still meets your own quality requirements.

Malt-Broth was inoculated with 100 µl of an overnight culture and incubated for 72 hours at 25°C – from the left:
1. negative control 2. *Saccharomyces cerevisiae* DSM 70449
3. *Candida tropicalis* DSM 70151 4. *Schizosaccharomyces pombe* DSM 70576

Overview about media for yeast and wort

Item	Item No	Recommendation for incubation
Beer-Agar	5015 (4 x 250 ml)	2-4 days at 30°C
Beer-NPS	1020 (50 tests) 1020-H (100 tests)	2-4 days at 30°C
BfB-Broth **for beer-spoiling microorganisms**	4008 (25 x 20 ml) 4008-100 (100 x 20 ml)	2-4 days at 30°C
BfW-Broth **for wild type yeasts**	4056 (25 x 20 ml) 4056-100 (100 x 20 ml)	2-6 days at 25°C
Brettanomyces-NPS	1025 (50 tests) 1025-H (100 tests)	4-7 days at 25°C
Copper Sulphate-Agar	5039 (4 x 250 ml)	2-5 days at 25°C
Crystal Violet-Agar	4051 (25 x 20 ml) 5038 (4 x 250 ml)	2-5 days at 25°C
Diastaticus-Agar	4027 (25 x 20 ml) 4027-100 (100 x 20 ml)	3-7 days at 25°C
Diastaticus-Broth	4029 (25 x 20 ml) 4029-100 (100 x 20 ml)	3-5 days at 25°C
Lysine-Agar	4055 (25 x 20 ml) 5042 (4 x 250 ml)	2-5 days at 25°C
Lysine-NPS	1095 (50 tests) 1095-H (100 tests)	2-5 days at 25°C
Malt-Broth	4058 (25 x 20 ml)	48-72 h at 25°C

Detailed technical data sheets are available at www.moeller-schmelz.de

Filling process and end product control

The goal of every brewery is to provide the consumer with maximum enjoyment with a safe drink. For this purpose, care must be taken during the filling process and in the final packaging that the beer does not contain any harmful or taste-impairing microorganisms such as wild yeast or lactic acid bacteria. To ensure this, both the filling lines and the end product are monitored.

The detection method for total germ count, coliform bacteria, beer spoilage, lactic acid bacteria, yeast and acetic acid bacteria corresponds to the test method for brewing water (see page 4-8).

Verification of the effectiveness of cleaning processes – Detection of indicator microorganisms and biofilms

As part of industrial hygiene, it is necessary to monitor the effectiveness of cleaning measures for systems, tanks, pipes and valves. With the **RV-Broth *item 4007*** developed by **Dr Möller & Schmelz**, indicator microorganisms from smears can be easily and reliably detected and classified.
If the result is positive, subsequent cleaning can be initiated quickly.

With the sterile swabs provided, a sample is taken from the area to be examined and transferred to the tube with RV-Broth. Incubation takes place aerobically for 24-48 hours at 36°C. A colour change from green to yellow (acid forming microorganisms) or to violet (microorganisms increasing the pH value) indicates a contamination.

RV-Broth was inoculated with 1 µl of an overnight culture and incubated aerobically for 24 hours at 36°C – from the left:
1. negative control 2. *Enterobacter faecalis* WDCM 00009
3. *Pseudomonas aeruginosa* WDCM 00024 4. *Escherichia coli* WDCM 00012

Media for monitoring the effectiveness of cleaning processes

Item	Item No	Recommendation for incubation
RV-Broth	4007 (25 x 10 ml)	24-48 h at 36°C
RV-Broth Kit	4017 (60 tests)	24-48 h at 36°C

Detailed technical data sheets are available at www.moeller-schmelz.de

Ordering information

Item	Item No	Package size
Beer-Agar	5015	4 x 250 ml
Beer-NPS	1020 / 1020-H	50 tests / 100 tests
BfB-Broth **for beer-spoiling microorganisms**	4008 4008-100	25 x 20 ml 100 x 20 ml
BfW-Broth for wild type yeasts	4056 / 4056-100	25 x 20 ml / 100 x 20 ml
Brettanomyces-NPS	1025 / 1025-H	50 tests / 100 tests
Colichrom-Agar	4028 / 4028-100 5025 / 5025-24	25 x 20 ml / 100 x 20 ml 4 x 250 ml / 24 x 250 ml
Colichrom-NPS	1035 / 1035-H	50 tests / 100 tests
Copper Sulphate-Agar	5039	4 x 250 ml
Crystal Violet-Agar	4051 5038	25 x 20 ml 4 x 250 ml
Diastaticus-Agar	4027 / 4027-100	25 x 20 ml / 100 x 20 ml
Diastaticus-Broth	4029 / 4029-100	25 x 20 ml / 100 x 20 ml
Lysine-Agar	4055 5042	25 x 20 ml 4 x 250 ml
Lysine-NPS	1095 / 1095-H	50 tests / 100 tests
Malt-Broth	4058	25 x 20 ml
MRS-Agar	4061 / 4061-100 5061 / 5061-24	25 x 20 ml / 100 x 20 ml 4 x 250 ml / 24 x 250 ml
MRS-NPS	1110 / 1110-H	50 tests / 100 tests
Nutrient-Agar	4080 / 4080-100 5080 / 5080-24	25 x 20 ml / 100 x 20 ml 4 x 250 ml / 24 x 250 ml
RV-Broth	4007	25 x 10 ml
RV-Broth Kit	4017	60 tests
Standard-Agar	4135 / 4135-100 5101 / 5101-24	25 x 20 ml / 100 x 20 ml 4 x 250 ml / 24 x 250 ml
Wort-Agar	4150 5110 / 5110-24	25 x 20 ml 4 x 250 ml / 24 x 250 ml
Wort-NPS	1260 / 1260-H	50 tests / 100 tests
Rinsing solution, for checking inside bottles	5128 5128-24	4 x 250 ml 24 x 250 ml
Rinsing solution, for filtration	5129 5129-24	4 x 250 ml 24 x 250 ml

Detailed technical data sheets are available at www.moeller-schmelz.de

Notes – Questions – Comments

Please do not hesitate to contact us: Phone +49 (0)551 6 67 08 | service@moeller-schmelz.de

Thank you very much, Your **M&S** Team

List of abbreviations

DIN	German Industrial Standard	**nm**	Nanometer
EN	European Standard	**µl**	Microliter
ISO	International Standardisation Organisation	**µm**	Micrometer
NPS	Nutrient Pad Set	**UV**	Ultraviolet

Disclaimer

The information contained in this brochure reflects our current level of knowledge and is only a general description of our products and possible applications. We do not assume any liability for the completeness, correctness, freedom from errors and appropriateness of this information and its use. It is the sole responsibility of the user to assess the suitability of the product for a particular application. We reserve the right to change this information and the product details at any time, in particular due to changes in legal provisions.

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