

## Technical Data Sheet

# Antibiotic Media (Antibiotic Agar No. 1, 5 and 11)

Ordering number: 1.05272.0500, 1.05271.0500, 1.05269.0500

For the microbiological assay of antibiotics in pharmaceutical preparations, body fluids, animal feed preparations, and other materials according to Grove and Randall (1955).

These culture media comply with the recommendations of the United States Pharmacopeia XXVI (2003) and the FDA. Antibiotic agar I also corresponds to medium A of the European Pharmacopeia II.

### Mode of Action

The sample material can be tested by dilution and diffusion methods. The most common method is the agar diffusion test which can be performed in various ways-cylinder, punched-hole or paper-disc tests. It is based on the following principle:

Antibiotic Agar No. 1	1.05272.0500	500 g
Antibiotic Agar No. 5	1.05271.0500	500 g
Antibiotic Agar No. 11	1.05269.0500	500 g

The culture medium is inoculated with the relevant test strain and poured into plates. Defined quantities of the antibiotic under examination and an antibiotic standard are applied as spots (cylinder, punched-hole, paper-discs). On incubation inhibition zones develop around the site of application, there is no microbial growth within these zones and their diameter is a measure of the activity of the antibiotic being tested. The activity of the antibiotic under test is determined by comparing the diameter of its inhibition zone with that of the antibiotic standard.

Carbohydrate and potato infusion (Beever and Bollard 1970) promote the growth of yeasts and molds while the low pH value partially inhibits the growth of the accompanying bacterial flora. If the medium is to be used for fungal counts, the pH should be adjusted to approximately 3.5. Fungi grow on this medium to develop typical morphology.

Product	Description	Standards
Antibiotic Agar No. 1		AOAC, EP, USP
Antibiotic Agar No. 2		AOAC, USP
Antibiotic Broth (Medium No. 3)		AOAC, EP, USP
Antibiotic Agar No. 4		AOAC, USP
Antibiotic Agar No. 5		AOAC, USP
Antibiotic Medium No. 6	can be prepared from Antibiotic Agar No. 2 and 1 g/litre D(+)glucose	
Antibiotic Agar No. 7	corresponds to Antibiotic Agar No. 2 but with pH 6.8-7.2	

Antibiotic Agar No. 8	corresponds to Antibiotic Agar No. 2 but with pH 5.7-6.1	AOAC, USP
Antibiotic Agar No. 9	can be prepared from CASO Broth, 20 g/l Agar-Agar	EP, USP
Antibiotic Agar No. 10	can be prepared from CASO Broth, 12 g/l Agar-Agar and 10 g/l Tween® 80	EP, USP
Antibiotic Agar No. 11		
Antibiotic Agar No. 12		
Sabouraud-2 % Dextrose Broth (Medium No. 13)		AOAC, USP

## Typical Composition

	Medium No. 1	Medium No. 2	Medium No. 3	Medium No. 4	Medium No. 5	Medium No. 6	Medium No. 7
Meat Extract	1.5 g/l	1.5 g/l	1.5 g/l	1.5 g/l	1.5 g/l	-	1.5 g/l
Yeast Extract	3.0 g/l	3.0 g/l	1.5 g/l	3.0 g/l	3.0 g/l	-	3.0 g/l
Peptone from Casein	4.0 g/l	-	-	-	-	17.0 g/l	-
Peptone from Meat	6.0 g/l	6.0 g/l	5.0 g/l	6.0 g/l	6.0 g/l	-	6.0 g/l
Peptone from Soymeal	-	-	-	-	-	3.0 g/l	-
D(+)-Glucose	1.0 g/l	-	1.0 g/l	1.0 g/l	-	2.5 g/l	-
NaCl	-	-	3.5 g/l	-	-	5.0 g/l	-
K <sub>2</sub> HPO <sub>4</sub>	-	-	3.68 g/l	-	-	2.5 g/l	-
KH <sub>2</sub> PO <sub>4</sub>	-	-	1.32 g/l	-	-	-	-
Agar-Agar	15.0 g/l	15.0 g/l	-	15.0 g/l	15.0 g/l	-	15.0 g/l
Polysorbate 80	-	-	-	-	-	-	-
Manganese sulfate	-	-	-	-	-	0.03 g/l	-

	Medium No. 8	Medium No. 9	Medium No. 10	Medium No. 11	Medium No. 12	Medium No. 13
Meat Extract	1.5 g/l	-	-	1.5 g/l	2.5 g/l	-
Yeast Extract	3.0 g/l	-	-	3.0 g/l	5.0 g/l	-
Peptone from Casein	-	17.0 g/l	17.0 g/l	4.0 g/l	-	-
Peptone from Meat	6.0 g/l	-	-	6.0 g/l	10.0 g/l	10 g/l
Peptone from Soymeal	-	3.0 g/l	3.0 g/l	-	-	-
D(+)-Glucose	-	2.5 g/l	2.5 g/l	1.0 g/l	10.0 g/l	20.0 g/l
NaCl	-	5.0 g/l	5.0 g/l	-	10.0 g/l	-



We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

Merck Millipore the M logo and Refobacin are registered trademarks of Merck KGaA, Darmstadt, Germany. ATCC is a registered trademark of ATCC, Manassas, VA, USA. TWEEN is a registered trademark of Croda International PLC Lit. No. TN7414EN00

K <sub>2</sub> HPO <sub>4</sub>	-	2.5 g/l	2.5 g/l	-	-	-
KH <sub>2</sub> PO <sub>4</sub>	-	-	-	-	-	-
Agar-Agar	15.0 g/l	20.0 g/l	12 g/l	15.0 g/l	25.0 g/l	-
Polysorbate 80	-	-	10.0 g/l	-	-	-
Mangane sulfate	-	-	-	-	-	-

## Preparation

Suspend the required quantity of culture medium (see Table). Autoclave 15 min at 121 °C. Add the test strain of bacteria at 45-50 °C. Pour plates.

The appearance of the ready-to-use plates is clear to slightly turbid and yellowish-brown.

The pH value at 25 °C is shown in the table below.

	Medium No. 1	Medium No. 2	Medium No. 3	Medium No. 4	Medium No. 5	Medium No. 6	Medium No. 7
Quantity required	30.5 g/l	25.5 g/l	17.5 g/l	26.5 g/l	25.5 g/l	30.0 g/l	25.5 g/l
pH at 25 °C	6.3-6.7	6.4-6.8	6.8-7.2	6.4-6.8	7.7-8.1	6.8-7.2	6.8-7.2

	Medium No. 8	Medium No. 9	Medium No. 10	Medium No. 11	Medium No. 12	Medium No. 13
Quantity required	25.5 g/l	50.0 g/l	52.0 g/l	30.5 g/l	62.5 g/l	30.0 g/l
pH at 25 °C	5.4-5.8	7.0-7.4	7.0-7.4	8.2-8.4	5.9-6.3	5.4-5.8

## Experimental Procedure and Evaluation

### 1. Cylinder test

*Procedure:* Fill Petridishes with 14 ml of the medium to form the base layer, after this has set overlay with 4 ml of the inoculated seed layer. Place steel or glass cylinders on the cooled culture medium under sterile conditions. The ready-to-use test plates can be stored in the refrigerator at 4°C. Pipette the antibiotic solutions into the cylinders and then incubate at 37 °C for 16-24 h.

*Evaluation:* Remove the cylinders, measure the diameters of the inhibition zones (it is best to use a "zone reading instrument") and evaluate them statistically. Draw a standard curve using the values of the standard solutions and read off the activities of the test solutions.

### 2. Punched-hole test

*Procedure:* Holes are punched out of the inoculated culture medium and the antibiotic solutions are then pipetted into them. All other steps are analogous to those described in the cylinder test.



We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

Merck Millipore the M logo and Refobacin are registered trademarks of Merck KGaA, Darmstadt, Germany. ATCC is a registered trademark of ATCC, Manassas, VA, USA. TWEEN is a registered trademark of Croda International PLC Lit. No. TN7414EN00

### 3. Paper-disc test

*Procedure:* Paper-discs with a diameter of 9 mm are impregnated with the antibiotic solution and placed on the culture medium. The antibiotic can also be applied to the disc after it has been placed on the medium. Plates containing a single layer of medium with a thickness of 2 mm can be used for these tests. Antibiotic agars nos. 2 or 5 may be employed depending on the pH required. All other steps are analogous to those described in the cylinder test.

### 4. Serial dilution test

The antibiotic activity is determined quantitatively by using the known sensitivity of a test strain towards an antibiotic which is expressed numerically as the minimal inhibitory concentration (MIC).

*Procedure:* Serial dilutions of the antibiotic to be tested are pipetted into the antibiotic broth, this is then inoculated with a defined quantity of the relevant test strain.

*Evaluation:* The last tube which does not show any turbidity due to microbial growth contains the active antibiotic at a concentration corresponding to the MIC.

### 5. Turbidimetric test

This test is more accurate and more sensitive than the serial dilution test.

*Procedure:* Incubate tubes containing 1 ml aliquots of the antibiotic solution and 9 ml aliquots of the inoculated antibiotic broth for 4 h at 37 °C in a water bath. The growth of the test bacteria is then stopped by adding 0.5 ml of a dilute formaldehyde solution and the turbidity evaluated photometrically.

*Evaluation:* The antibiotic concentration is determined by comparing the absorbance of the test solution with that of a previously constructed standard curve.

### Use of Antibiotic Culture Media

Antibiotic		Cylinder Test			Turbidimetric Test	
			Culture Medium		Test Strain	Culture Medium
	Test Strain	Seed Culture	Base Layer	Seed Layer		
Amphotycin	<i>Micrococcus luteus</i> ATCC 14452	Medium No. 1	Medium No. 7	Medium No. 1	-	-
Amphotericin B	<i>Saccharomyces cerevisiae</i> ATCC 9763	Medium No. 13	Medium No. 12	Medium No. 12	-	-
Ampicillin	<i>Micrococcus luteus</i> ATCC 9341	Medium No. 1	Medium No. 11	Medium No. 11	-	-
Bacitracin	<i>Micrococcus luteus</i> ATCC 10240 <i>Micrococcus luteus</i> ATCC 7468 D	Medium No. 1	Medium No. 2	Medium No. 1	<i>Staphylococcus aureus</i> ATCC 10537	Medium No. 3
Carbomycin	<i>Micrococcus luteus</i> ATCC 9341	Medium No. 3	Medium No. 11	Medium No. 11	-	-
Chloramphenicol	<i>Micrococcus luteus</i> ATCC 9341	Medium No. 3	Medium No. 1	Medium No. 1	-	-



We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

Merck Millipore the M logo and Refobacin are registered trademarks of Merck KGaA, Darmstadt, Germany. ATCC is a registered trademark of ATCC, Manassas, VA, USA. TWEEN is a registered trademark of Croda International PLC Lit. No. TN7414EN00

Cephalothin	<i>Staphylococcus aureus</i> ATCC 6538 P	Medium No. 1	Medium No. 2	Medium No. 1	-	-
Colistin	<i>Bordetella bronchiseptica</i> ATCC 4617	Medium No. 9	Medium No. 9	Medium No. 10	-	-
Erythromycin	<i>Micrococcus luteus</i> ATCC 9341	Medium No. 3	Medium No. 11	Medium No. 11	-	-
Gentamicin (Refo-bacin® Millipore Sigma)	<i>Bacillus subtilis</i> ATCC 6633	Medium No. 1	Medium No. 5	Medium No. 5	-	-
Kanamycin	<i>Staphylococcus aureus</i> ATCC 6538 P	Medium No. 1	Medium No. 11	Medium No. 11	<i>Staphylococcus aureus</i> ATCC 6538 P	Medium No. 3
Neomycin	<i>Staphylococcus aureus</i> ATCC 6538 P	Medium No. 1	Medium No. 11	Medium No. 11	-	-
Novobiocin	<i>Staphylococcus epidermidis</i> ATCC 12228	Medium No. 1	Medium No. 2	Medium No. 1	-	-
Oleandomycin	<i>Staphylococcus epidermidis</i> ATCC 12228	Medium No. 1	Medium No. 11	Medium No. 11	-	-
Paromomycin	<i>Staphylococcus epidermidis</i> ATCC 12228	Medium No. 1	Medium No. 11	Medium No. 11	<i>Klebsiella pneumoniae</i> ATCC 10031	Medium No. 3

Antibiotic		Cylinder Test			Turbidimetric Test	
			Culture Medium		Test Strain	Culture Medium
	Test Strain	Seed Culture	Base Layer	Seed Layer		
Polymyxin B	<i>Bordetella bronchiseptica</i> ATCC 4617	Medium No. 9	Medium No. 9	Medium No. 10	-	-
Penicillin, oxacillin, methicillin, nafcillin	<i>Staphylococcus aureus</i> ATCC 6538 P	Medium No. 3	Medium No. 2	Medium No. 1	-	-
Streptomycin, dihydrostreptomycin	<i>Bacillus subtilis</i> ATCC 6633	Medium No. 1	Medium No. 5	Medium No. 5	<i>Klebsiella pneumoniae</i> ATCC 10031	Medium No. 3
Tetracycline, oxytetracycline, chlorotetracycline	<i>Bacillus cereus</i> ATCC 1178	Medium No. 1	Medium No. 8	Medium No. 8	<i>Staph. aureus</i> ATCC 6538 P	Medium No. 3
Viomycin	<i>Bacillus subtilis</i> ATCC 6633	Medium No. 1	Medium No. 5	Medium No. 5	-	-



We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

Merck Millipore the M logo and Refobacin are registered trademarks of Merck KGaA, Darmstadt, Germany. ATCC is a registered trademark of ATCC, Manassas, VA, USA. TWEEN is a registered trademark of Croda International PLC Lit. No. TN7414EN00

Manufacturer	Product
American Type Culture Collection 12301 Parklawn Drive, Rockville Maryland 20852, USA	Test strains
USP Reference Standards 4630 Montgomery Avenue Bethesda, MD 20014, USA	Antibiotic Standards
Schleicher & Schüll BioScience GmbH 37586 Dassel, GER	Paper-discs No. 2628

## Quality Control

### Antibiotic Agar No. 1

Control Strains	ATCC #	Incubation	Growth	Inhibition Zones with
<i>Kocuria rhizophila</i>	9341	24 h at 35 °C	Good to very good	Cephalotin, Penicillin, Chloramphenicol
<i>Staphylococcus aureus</i>	6538 P	24 h at 35 °C	Good to very good	Cephalotin, Penicillin, Chloramphenicol
<i>Bacillus subtilis</i>	6633	24 h at 35 °C	Good to very good	
<i>Staphylococcus epidermidis</i>	12228	24 h at 35 °C	Good to very good	
<i>Bacillus cereus</i>	11778	24 h at 35 °C	Good to very good	

Please refer to the actual batch related Certificate of Analysis.

### Antibiotic Agar No. 5

Control Strains	ATCC #	Incubation	Growth	Inhibition Zones with
<i>Bacillus subtilis</i>	BGA	24 h at 35 °C	Good to very good	Streptomycin, Gentamycin

Please refer to the actual batch related Certificate of Analysis.

### Antibiotic Agar No. 11

Control Strains	ATCC #	Incubation	Growth	Inhibition Zones with
<i>Kocuria rhizophila</i>	9341		Fair to good	Ampicillin, Erythromycin
<i>Staphylococcus aureus</i>	6538 P		Good to very good	Kanamycin Neomycin
<i>Staphylococcus epidermidis</i>	12228		Good to very good	Oleandomycin, Neomycin

Please refer to the actual batch related Certificate of Analysis.



We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

Merck Millipore the M logo and Refobacin are registered trademarks of Merck KGaA, Darmstadt, Germany. ATCC is a registered trademark of ATCC, Manassas, VA, USA. TWEEN is a registered trademark of Croda International PLC Lit. No. TN7414EN00

## Literature

Abraham, E.P., Chain, E., Fletcher, C.M., Florey, H.W., Gardner, A.D., Heatley, N.G. and Jennings, M.A. (1943). Further observations on penicillin. *Lancet*, **241**: 177-189.

European Pharmacopeia II (2003). Chapter VIII, 4.

Forster, J.W. and Woodruff, H.B. (1943). Microbial aspects of penicillin. *J. Bact.* **46**: 187-202.

Grove, D.C. and Randall, W.A. (1955). Assay Methods of antibiotics. *Medical Encyclopedia*, N.Y.

Schmidt, H.W. and Moyer, A.J. (1944). Penicillin I. Methods of assay. *J. Bact.*, **47**; 199-208.

United States Pharmacopeia XXVI (1995). Chapter "Biological Tests and Assays".

Wallhäuser, K.H. und Schmidt, H. (1967). *Sterilisation, Desinfektion, Konservierung, Chemotherapeutica*. G. Thieme-Verlag, Stuttgart.

## Ordering Information

Product	Cat. No.	Pack size
Antibiotic Agar No. 1	1.05272.0500	500 g
Antibiotic Agar No. 5	1.05271.0500	500 g
Antibiotic Agar No. 11	1.05269.0500	500 g
Sabouraud-2 % Dextrose Broth (Medium No. 13)	1.08339.0500	500 g
D(+)Glucosemonohydrate	1.08342.1000	1 kg
Agar-Agar purified	1.01614.1000	1 kg
Manganese(II) Sulfate Monohydrate	1.05999.0500	500 g
Tween® 80	8.22187.0500	500 ml
Tryptic Soy Broth	1.05459.0500	500 g

Merck KGaA, 64271 Darmstadt, Germany  
Fax: +49 (0) 61 51 / 72-60 80  
mibio@merckgroup.com  
[www.merckmillipore.com/biomonitoring](http://www.merckmillipore.com/biomonitoring)

Find contact information for your country at:  
[www.merckmillipore.com/offices](http://www.merckmillipore.com/offices)  
For Technical Service, please visit:  
[www.merckmillipore.com/techservice](http://www.merckmillipore.com/techservice)



We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

Merck Millipore the M logo and Refobacin are registered trademarks of Merck KGaA, Darmstadt, Germany. ATCC is a registered trademark of ATCC, Manassas, VA, USA. TWEEN is a registered trademark of Croda International PLC Lit. No. TN7414EN00