

Technical Data Sheet

Test Agar pH 8.0 for the Inhibitor Test

Ordering number: 1.10664.0500

For the detection of antimicrobial inhibitors in meat and organ samples together with *Bacillus subtilis* (BGA) spore suspension and *Micrococcus luteus* ATCC 9341 as test organisms.

The nutrient media are suitable both, for the inhibitor test (LEVETZOW, 1971) according to the German Meat Inspection Law as well as for the EEC Four-Plate-Test (BOGAERTS and WOLF, 1980) suggested by the Scientific Veterinary Commission of the European Economic Community.

Test agar pH 7.2 with addition of trimethoprim is used particularly for determination of sulfonamide residues.

Mode of Action

The test is carried out according to the agar diffusion procedure. Small slices of the meat sample are placed on the inoculated Test Agar plates and incubated. Antimicrobial inhibitors contained in the samples diffuse into the nutrient media and cause growth-free inhibition zones to develop on the otherwise thickly covered plates. Repeated tests with pH 6.0, pH 8.0 and pH 7.2 are necessary, as penicillin and streptomycin are optimally active at pH 6.0 and 8.0 respectively (PICHNARCIK et al., 1969) and the activity optimum of sulfonamide is found at pH 7.2. Addition of trimethoprim to Test Agar pH 7.2 considerably increases the sensitivity of the test system to sulfonamides (GUDDING, 1976; EBRECHT, 1982).

Typical Composition (g/L)

Test Agar pH 8.0 for the Inhibitor Test	
Peptone from casein, tryptic	3.45
Peptone from meat, tryptic	3.45
Sodium chloride	5.1
Tri-sodium phosphate 12- hydrate	2.4
Agar-agar**	13.0

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

Preparation

Suspend 27.5 g/litre Test Agar pH 8.0, autoclave (15 min at 121 °C), test the pH and if necessary adjust. Cool to 50-45°C, mix in 1 ml/litre *Bacillus subtilis* (BGA) spore suspension. If necessary, add *Micrococcus luteus* ATCC 9341 (microbial count in culture medium: approximately 10⁴ cfu/ml) to Test Agar pH 8.0.

After mixing the spore suspension, immediately pour the plates and place in the refrigerator.

The plates are clear and yellowish-brown.

Experimental Procedure and Evaluation

Inhibitor Test

Test Agar pH 8.0 with *Bacillus subtilis* (BGA)

EEC Four-Plate-Test

Test Agar pH 8.0 with *Bacillus subtilis* (BGA) and Test Agar pH 8.0 with *Micrococcus luteus* ATCC 9341.

For details regarding the collection of samples, transportation as well as the execution of the test, see the Fleischbeschaugesetz (Meat Inspection Law) or BOGAERTS and WOLF (1980).

According to these specifications the cylinder-shaped tissue sections (8 mm in diameter and 2 mm thick) are stamped out under conditions, which are as aseptic as possible and laid on the plates; according to BOGAERTS and WOLF two sections are required per plate. As a control, one test disc with 10 IU of penicillin G-sodium is placed on a plate with pH 6.0, one test disc with 10 µg of streptomycin on each of the two plates with pH 8.0 and one test disc with 0.5 µg sulfadimidine on a plate with pH 7.2. The test discs can also be made by the user using filter paper discs, 6 mm in diameter.

Incubation: 18-24 hours at 30°C (*Bacillus subtilis* BGA) and 37 °C (*Micrococcus luteus* ATCC 9341).

The inhibition zones between the edge of the tissue section or the test disc and the growth limit of the test organism are measured. Complete inhibition of growth with an inhibitory zone of at least 2 mm is regarded as positive result, an inhibitory zone of 1-2 mm should be considered as doubtful. This only applies, however, if the controls, prepared at the same time, display zones of inhibition measuring about 6 mm.

For possible methodological improvements see FORSCHNER and SEIDLER (1976).

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.

The ready-to-use plates can be sealed with air-tight adhesive tape and stored refrigerated at 4 - 6°C for potentially up to 2 weeks. Additional packing into plastic bags is recommended. If stored for longer periods, the temperature should not be higher than 3°C. Freezing of the culture medium must be avoided.



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Quality Control

Parameter	Diameter	Growth
Inhibition zone diameter Gentamicin 10 µg Bacillus subtilis BGA	32-42 mm	
Inhibition zone diameter Gentamicin 30 µg; Bacillus subtilis BGA	35-45 mm	
Inhibition zone diameter Penicillin G 10 U/IE Bacillus subtilis BGA	40-48 mm	
Inhibition zone diameter Streptomycin 10 µg Bacillus subtilis BGA	30-36 mm	
Bacillus subtilis BGA		good to very good
Kocuria rhizophila ATCC 9341		good to very good

Please refer to the actual batch related Certificate of Analysis.



Bacillus subtilis BGA



Micrococcus luteus ATCC 9341



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Literature

Arbeitsgruppe des Instituts für Lebensmitteltechnologie und Verpackung der Technischen Universität München: Merkblätter für die Prüfung von Packmitteln, Merkblatt 18 "Prüfung auf antimikrobielle Bestandteile in Packstoffen". - **Verpackgs.-Rdsch.**, **25**; Techn.-wiss. Beilagen; 5-8 (1974).

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Ordering Information

Product	Cat. No.	Pack size
Test Agar pH 8.0 for the Inhibitor Test	1.10664 .0500	500 g
Bacillus subtilis (BGA)-spore suspension	1.10649.0001	15 x 2 ml
Penicillin G, Potassium Salt	Calbiochem 5161-25MU	25 MU
Gentamycin sulfate	Calbiochem 345814-1GM	1 g
Streptomycin sulfate	Calbiochem 5711-100GM	100 g

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