

Bacillus Subtilis (BGA) Spore Suspension

For the inhibitor test.

Bacillus subtilis (BGA) spore suspension is used with the culture media test agar pH 6.0 for the inhibitor test Cat. No. 1.10663. and test agar pH 8.0 for the inhibitor test Cat. No. 1.10664. for the detection of antimicrobial inhibitors in meat by routine methods.

Mode of Action

The test is conducted as an agar diffusion test. The spores of bacillus subtilis (BGA) are used as the test organisms. Inhibitors inhibit the growth of the test bacteria. The inhibition of growth is indicated by inhibition zones.

Equipment

Autoclave or pressure cooker, incubation cabinet.

Auxilliaris

Petridish or other dish with lid for nutrient media.

Reagents

Bacillus subtilis (BGA) spore suspension, adjusted to a content of 10^7 CFU/ml (range: 8×10^6 to 5×10^7 CFU/mg).

Test agar pH 6.0 for the inhibitor test Cat. No. 1.10663.

Composition	(g per litre)
Peptone from casein	3.45
Peptone from meat	3.45
Sodium chloride	5.1
Agar agar	13.0

Test agar pH 8.0 for the inhibitor test Cat. No. 1.10664.

Composition	(g per litre)
Peptone from casein	3.45
Peptone from meat	3.45
Sodium chloride	5.1
Phosphate buffer	2.4
Agar agar	13.0

Preparation of the Ready-to-use Test Agar

Suspend 25 g/l (test agar pH 6.0) or 27.5g/litre (test agar pH8.0) in freshly distilled or fully demineralized water. Then boil in a pressure cooker until completely dissolved. The sterilization is carried out in an autoclave (15 minutes at +121°C).

Cool to +50 °C, then add 1ml bacillus subtilis (BGA) spore suspension per litre of nutrient medium and shake. Pour 15ml of the nutrient medium into each Petridish.

Storage of the Ready-to-use Test Agar

The ready-to-use test agar can be sealed into Petridishes with airtight adhesive tape and stored in a refrigerator (+2 to +8 °C) for up to 2weeks. Additional packaging in plastic bags is recommended.

Carrying out the Test

For instructions on taking and sending samples as well as conducting the test, refer to the regulations on the inspection of meat products.

Stamp out cylindrical disks of tissue with a diameter of 8mm and a thickness of 2mm under clean conditions avoiding contamination and place one on a plate of pH6.0 and one on a plate of pH8.0. As a control, place a test strip with 0.01IU penicillin G-sodium on a pH6.0 plate and one test strip with 0.5µg streptomycin on a pH8.0 plate.

Incubation: 18 to 24 hours at +30°C.

Evaluation

Measure the inhibition zone between the edge of the piece of tissue and the limit of growth. Complete inhibition of growth with an inhibition zone of at least 2mm can be regarded as a positive result, an inhibition zone of 1 to 2mm as a dubious result, if the parallel controls have inhibition zones of approx. 6mm.

Storage of Bacillus Subtilis (BGA) Spore Suspension

Storage at +2 to +8 °C in a refrigerator is recommended. Storage at room temperature (up to +25 °C) is only possible for 1 to 2days, otherwise the stability is adversely affected.

Stability

Only with proper storage in a refrigerator the stability can be guaranteed up to the expiry date given. Thereafter, the activity of the spores must be expected to begin to decline.

Literature

Levetzow, R.: Untersuchungen auf Hemmstoffe im Rahmen der Bakteriologischen Fleischuntersuchung (BU). - **Bundesgesundheitsblatt**, 1971; **14**: **15/16**, 211-213.

Ordering Information

Product	Merck Cat. No.	Pack contents
Bacillus Subtilis (BGA) Spore Suspension	1.10649.0001	15 x 2ml ampoules