

Test Agar for the Residue Test acc. to KUNDRAT

Medium introduced by KUNDRAT (1968, 1972) for the routine qualitative detection of residues of antibiotics, sulfonamides and other chemotherapeutic agents in meat and other foodstuffs derived from animals.

Version: 03.03.2011

General Information

The test is carried out using a spore suspension of *Bacillus geothermophilus* (MERCK, Cat. No. 1.11499.) as test micro-organisms. Cleaning agents, disinfectants and preservatives are not covered by this test. The medium can be used for rapid or long-term tests.

Mode of Action

The test is performed in the form of an agar diffusion test. Any inhibitors present produce inhibition zones devoid of bacterial growth surrounding the applied samples. With further incubation, the test organism ferments glucose present in the medium to form acid; this causes bromocresol purple to change its colour to yellow. Only the inhibition zones still retain the original violet colour of the indicator. When performing the rapid test, growth of the test organism is enhanced by pre-incubating the inoculated plates; the inhibition zones then appear more rapidly after application of the samples.

Typical Composition (g/litre)

Peptone 17.0; sodium chloride 3.0; D(+)glucose 3.0; starch 3.0; gelatin 2.5; bromocresol purple 0.016; sucrose 2.0; agar-agar 10.0.

Preparation

Suspend 40.5 g/litre and autoclave (15 min at 121 °C). Cool to 50-60 °C, to each 200 ml add the contents of 1 ampoule of *Bacillus geothermophilus* spore suspension ATCC 7953 (sporulation optimized), mix, pour plates. Acc. to the German DIN 10182 part 1, a volume of 5 ml/Petridish (Ø 90 mm) is recommended.

pH: 6.8 ± 0.2 at 25 °C.

The plates are clear and purple.

Plates which are to be used for the rapid test should be pre-incubated for 135 minutes at 65 °C. The plates should not be stacked in order to ensure even temperature.

Storage recommendations

- If the ready-to-use plates are sealed with air-tight adhesive tape, they may be stored for up to 3 months in the refrigerator. The pre-incubated plates can be stored for up to one month under the same conditions. It is also advised to put the sealed plates into plastic bags.

Experimental Procedure and Evaluation

Filter paper discs with a diameter of 6 mm are soaked with the liquid specimen or placed on organ (kidney, liver) or muscle sections. The discs are then slightly pressed onto the surface of the culture medium (up to 6 discs per plate).

Two methods are recommended for performing the test:

1. 45 minutes incubation, rapid test: After placing the discs on the pre-incubated plates, incubate them for a further 45 minutes at 65 °C without pre-diffusion.
2. 3 hour incubation: The plates are not pre-incubated. After the filter paper discs have been applied to the plates, they should be incubated for 3 hours at 65 °C without prediffusion.

In the case of rapid test, formation of inhibition zones can be seen after 15-25 minutes incubation in the medium, which is otherwise turbid as a result of spore growth. After the 45 minutes incubation, the inhibition zones become even more distinct due to the fact that the culture medium changes colour. Formation of inhibition zones is to be regarded as a positive result.

In the case of the 3 hours incubation, only those inhibition zones with a diameter of more than 10 mm can be considered positive.

If a distinct colour change has not occurred after 45 minutes or 3 hours, incubation can be prolonged.

Literature

KUNDRAT, W.: Methoden zur Bestimmung von Antibiotika-Rückständen in tierischen Produkten. - Z. Anal. Chem.; 624-630 (1968).

KUNDRAT, W.: 45-Minuten-Schnellmethode zum mikrobiologischen Nachweis von Hemmstoffen in tierischen Produkten. - Fleischwirtsch., 52; 485-487 (1972).

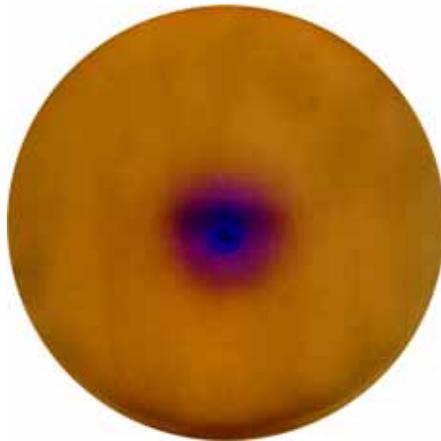
Ordering Information

Product	Ordering No.	Pack size
Test Agar for the Residue Test acc. to KUNDRAT	1.10662.0500	500 g
<i>Bacillus geothermophilus</i> spore suspension	1.11499.0001	5 x 2 ml

Test Agar for the Residue Test acc. to KUNDRAT

Quality control

Test strains	Growth after 3-3.5 h at 65 °C	Colour change to yellow	Inhibition zones in mm Ø			
			Gentamicin 10 µg	30 µg	Penicillin 10 IU	Streptomycin 10 µg
<i>Bacillus geothermophilus</i> ATCC 7953	good / very good	+	18-24	20-26	35-40	14-21



Genamycin 30 µg



Streptomycin 10 µg