Mineral-modified glutamate agar (MMGA) acc. to ISO 16649

General Information

Culture medium for the resuscitation of damaged *Escherichia coli* from foods.

In combination with Chromocult® TBX agar, the enumeration of *E. coli* is possible using the membrane filter method corresponding to ISO 16649-1.

This membrane filter method with the resuscitation step is particularly suitable in the case of food treatment may result in damage to the target organisms (for example heating, drying, chemical preservation, freeze-drying, acidification).

Mode of Action

The combination of glutamate with selected minerals results in very rapid and effective resuscitation of damaged *E. coli*. After incubation for 4 hours at 37 °C, the bacteria repair process is advanced that growth on a selective culture medium (Chromocult® TBX agar) at 44 °C is guaranteed.

Typical Composition (g/l)

Sodium glutamate 6.35; lactose 10.0; sodium formate 0.25; L-cystine 0.02; L-aspartic acid 0.02; L-arginine monohydrochloride 0.029; thiamine dichloride 0.001; nicotinic acid 0.001; calcium D pantothenate 0.001; magnesium sulfate heptahydrate 0.1; ammonium iron(III) citrate 0.01; calcium chloride dihydrate 0.01; di-potassium hydrogen phosphate 0.9, ammonium chloride 2.5; agar-agar 10.0.

Preparation

Boil 15.1 g in 500 ml of demineralized water in a boiling water bath or in a current of steam with regular swirling until the culture medium has completely dissolved.

Autoclave under mild conditions (10 minutes at 115°C). Excessive heating causes a change in pH.

Cool the culture medium to 45 - 50°C in the water bath and pour plates (12 - 15 ml).

pH: 6.7 ± 0.2 at 25°C.

The prepared culture medium is slightly yellowish and clear.

Storage

The prepared plates have a shelf life of 5 days when stored appropriately (+2°C to +8°C) - protect against drying-out and light.

Package can be used up to the expiration date if stored dry and well sealed at +15°C to +25°C.

Experimental Procedure and Evaluation

Bring the plates to room temperature.

Dry the plates immediately before use. The agar should be dry enough to allow excess moisture to disappear within 15 min of spreading the inoculum of 1 ml.

Inoculation:

Place a cellulose mixed ester membrane filter (e.g. Sartorius cellulose nitrate filter #11306-85-K, Pall GN-6 Metricel P/N 60016- in each case with a pore size of 0.45 µm and a diameter of 85 mm) on the agar surface using sterile tweezers. Avoid air bubbles.

Place 1 ml of the sample in the centre of the membrane using a sterile pipette and distribute uniformly over the membrane using a sterile spatula. Do not damage the membrane. 2 plates are inoculated per dilution.

Leave the inoculated plates to stand in the horizontal position for about 15 minutes at room temperature until the inoculum has been completely absorbed.

Resuscitation (MMGA):

4 ± 1 hours at 35 - 37°C with the membrane on top.

Transfer to selective medium:

Carefully lift the membrane off the MMGA using sterile tweezers and place on the Chromocult® TBX agar without the formation of air bubbles.

Incubation:

21 ± 3 hours at 44 ± 1°C with the membrane on top. Do not stack more than 3 plates on top of one another.

Evaluation (Chromocult® TBX agar):

All colonies which appear blue-green on the membrane are counted as β-glucuronidase-positive *E. coli* colonies (typical colonies).

Select the dilution of the sample so that the number of typical *E. coli* colonies on the membrane does not exceed 150 or the total colony count does not exceed 300 colonies.

Literature

ISO INTERNATIONAL STANDARDISATION ORGANISATION Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli*. Part 1: Colony-count technique at 44°C using membranes and 5-bromo-4-chloro-3-indolyl-β-D-glucuronide. ISO 16649-1:2001 (E).

Ordering Information

Product	Ordering No.	Pack size
Mineral-modified glutamate agar in accordance with ISO 16649	1.09045.0500	500 g
Chromocult® TBX agar	1.16122.0500	500 g

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Quality control

Test strains	Heat damage 55 °C	Resuscitation MMGA	Recovery rate TBX	Colony colour TBX
Escherichia coli ATCC 25922	2-5 min	4 h	> 70%	blue-green
Escherichia coli ATCC 11775	2-5 min	4 h	> 70%	blue-green
Citrobacter freundii ATCC 8090	2-5 min	4 h	< 0.001%	-
Enterococcus faecalis ATCC 19433	2-5 min	4 h	< 0.001%	-