

TECHNICAL DATA SHEET

Article No. 9842

Kanamycin Esculin Azide Agar, ready-to-use culture medium

SYNONYMS

KAA-Agar, KAA Confirmatory Agar

SPECIFICATION

Prepared medium. Solid medium for confirmative detection and isolation of Lancefield's group D streptococci in food samples, according to Mossel et al.

Colour: Olive brown
pH: 7.0 ± 0.2 at 25 °C

COMPOSITION IN G/L

Tryptone	20.00
Yeast extract	5.00
Sodium chloride	5.00
Sodium citrate	1.00
Esculin	1.00
Ammonium ferric citrate	0.50
Sodium azide	0.15
Kanamycin	0.02
Agar	15.00

PACKAGING DETAILS

9842-10x200ML

10 prepared bottles

Content: 200 ± 5 ml

Packaging unit: 1 box with 10 bottles 250 ml. Plastic screw inner cap. For use with syringe needles with a diameter ≤ 0.8 mm.



GUIDELINES

Description:

Kanamycin Esculin Azide Agar (KAA) confirmative Agar is a medium that several organizations and institutes recommend for detecting, enumerate and isolate Lancefield's group D streptococci in samples of food and beverages e.g.: bottled water, fresh/refrigerated/frozen/minced meat, fish, mollusks, soft drinks, pastries and spices. Kanamycin and sodium azide are the selective inhibitory compounds.

Technique:

Melt the medium contained in the bottles in a water bath (100 °C) or in a microwave oven, avoiding overheating before pouring into petri dishes when cooled to room temperature.

From samples considered positive, aliquots of 0,1 ml are inoculated onto the surface of the plates of KAA, spreading with a drigalsky loop. Incubate the plates, in an inverted position, at 37 °C for 24 hours. Colonies that appear surrounded by a black halo are considered as group D streptococci, and are isolated to confirm them biochemically and morphologically with the following tests: microscopical examination; catalase assay (that should be negative) in an azide-less medium; growth at 45 °C and resistance to a high saline concentration 6,5 % of NaCl in BHI Broth .

Finally, they have to grow in Bile Esculin Agar with an appearance similar to the colonies on the KAA Confirmative Agar. Nonetheless, there are some exceptions to this rule, i.e. *Streptococcus equinus* and *Streptococcus bovis* do not grow in the hypersaline broth, and therefore, definitive identification has to be performed by serological methods.

This methodology does not allow the enumeration of bacteria from the original sample, and as this is a necessary, the Most Probable Number (MPN) technique is recommended with KAA Presumptive Broth, using double strength broth if necessary.

Note: The solid media can be melted in different ways: autoclave, bath and, if the customer considers appropriate, also the microwave. Whenever the microwave option is chosen, it is necessary to take certain safety measures to avoid breaking of the containers, such as loosening the screw cap and putting the bottle or tube in a water bath in the microwave. The fusion temperature and time will depend on the shape of the container, the volume of medium and the heat source. Avoid overheating as both the heating periods.

MICROBIOLOGICAL CONTROL

Melt the medium and inoculate 10³-10⁴ CFU (Productivity test qualitative)/ 10⁴-10⁶ CFU (Selectivity)

Microbiological control according to ISO 11133:2014/A1:2018.

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020

Aerobiosis. Incubation at 36 ± 2 °C, reading at 44±4 h

Microorganism	Growth
<i>Enterococcus faecalis</i> ATCC® 29212, WDCM 00087	Good. Brown to black colonies. Positive esculin.
<i>Enterococcus faecalis</i> ATCC® 19433, WDCM 00009	Good. Brown to black colonies. Positive esculin.
<i>Escherichia coli</i> ATCC® 25922, WDCM 00013	Inhibited
<i>Staphylococcus aureus</i> ATCC® 25923, WDCM 00034	Inhibited

Sterility control:

Incubation 48 hours at 30-35 °C and 48 hours at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

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STORAGE

8-25 °C

SHELF LIFE

12 months unopened from date of manufacture

updated: 30.08.2022

