

Kanamycin Sulphate Supplement

PRODUCT INFORMATION

K008-1g - Kanamycin Sulfate, Powder, 1g

K008-5g - Kanamycin Sulfate, Powder, 5g

K008-25g - Kanamycin Sulfate, Powder, 25g

K013-1g - Kanamycin A Sulfate, EvoPure, Powder, 1g

K013-5g - Kanamycin A Sulfate, EvoPure, Powder, 5g

K002-100mg - Kanamycin B Sulfate, Powder, 100mg

K002-250mg - Kanamycin B Sulfate, Powder, 250mg

K002-1g - Kanamycin B Sulfate, Powder, 1g

K014-250mg - Kanamycin B Sulfate, EvoPure, Powder, 250mg

K014-1g - Kanamycin B Sulfate, EvoPure, Powder, 1g

K004-1g - Kanamycin Disulfate, Powder, 1g

K004-5g - Kanamycin Disulfate, Powder, 5g

K004-25g - Kanamycin Disulfate, Powder, 25g

DESCRIPTION

Kanamycin Aesculin Azide Agar Base with Kanamycin Sulphate Supplement is a selective medium for the isolation of *enterococci* in foodstuffs.

BACKGROUND

Kanamycin sulfate is an aminoglycoside antibiotic. Kanamycin is isolated from *Streptomyces kanamyceticus*.

Mechanism of action

APPLICATION IN KANAMYCIN AESCULIN AZIDE AGAR BASE

Kanamycin Aesculin Azide Agar was designed by

Mossel et al. to detect *enterococci* in foodstuffs. Round, white or grey colonies about 2 mm in diameter, surrounded by black zones of at least 1 cm diameter are considered to be *enterococci* (presumptive). Incubation is carried out aerobically at 35°C or 42°C ± 0.3°C for 18-24 hours. The higher incubation temperature increases the selectivity of the medium. This medium was used by Mossel et al. in the Dip Slide technique for bacteriological monitoring of foods. Kanamycin Aesculin Azide Agar has been used successfully for the isolation of glycopeptide-resistant *enterococci* from clinical specimens and foods.

Kanamycin Aesculin Azide Agar Base replaced Kanamycin Aesculin Azide Agar to follow the rules that antibiotics should not be present in powdered culture media where they can be inhaled or contaminate surfaces. Kanamycin sulphate is added separately to reconstituted agar from freeze-dried vials Kanamycin Sulphate Supplement containing the precise amount of antibiotic required.

The medium contains the selective inhibitory components kanamycin sulphate and sodium azide. It also contains an indicator system to detect the growth of aesculin-hydrolysing *streptococci*. These organisms produce black zones around the colonies from the formation of black iron phenolic compounds derived from aesculin-hydrolysis products and ferrous iron.

Content concentrations

Typical Formula*	mg/litre
Kanamycin Aesculin Azide Agar Base	
Tryptone	20
Yeast extract	5
Sodium chloride	5
Sodium citrate	1
Aesculin	1
Ferric ammonium citrate	0.5
Sodium azide	0.15
Agar	10
Final pH 7.0 ± 0.2 @ 25°C	
Kanamycin Sulphate Supplement	
Kanamycin sulphate	20.0
* Adjusted as required to meet performance standards	

Table 1 - Typical Formula for Kanamycin Aesculin Azide Agar Base and Kanamycin Sulphate Supplement

METHOD

Preparation

Suspend appropriate amount of Kanamycin Aesculin Azide Agar Base in distilled water. Add Kanamycin Supplement reconstituted as directed. Bring to the boil. Sterilise by autoclaving at 121°C for 15 minutes.

Protocol

Inoculation method for samples: spread 0.1 ml of sample dilutions over the whole of a pre-dried 9 cm diameter plate.

The following procedure for testing foodstuffs is adapted from Mossel, Harrewijn and Elzebroek.

1. Prepare tubes of sterilised tryptone water in 9 ml volumes. Chill to 0-5°C by storing in a refrigerator for 18 hours prior to use.
2. Add 1 g or 1 ml of the thoroughly mixed food sample to a tube containing 9 ml of pre-chilled diluent (10-1 dilution). Shake well for 30 seconds. Sample 1 ml of the contents, within 30 seconds after mixing, into a fresh tube of diluent. Continue the process using fresh sterile pipettes until a dilution is reached which will produce 100 colonies per 1 ml. Store the decimal dilutions in the refrigerator and examine within 3 hours of their preparation.
3. Streak onto plates of Kanamycin Aesculin Azide Agar and incubate for 16-24 hours at 35°C ± 1°C. Consider the result positive for *enterococci* when colonies surrounded by black haloes are grown.
4. Confirmatory tests may be carried out, e.g. catalase test, utilisation of glucose, growth at 45°C ± 1°C, chain-forming Gram-positive cocci.

Quality control

Positive control:

Enterococcus faecium ATCC® 19434: Good growth; black colonies and aesculin hydrolysis

Streptococcus bovis ATCC® 27960: Good growth; black colonies

Negative control:

Bacillus subtilis ATCC® 6633: No growth

Escherichia coli ATCC® 25922: Inhibited

REFERENCES

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