

KANAMYCIN AESCULIN AZIDE AGAR BASE KANAMYCIN SELECTIVE SUPPLEMENT

Dehydrated culture medium and selective supplement

1 - INTENDED USE

Selective and differential basal medium and selective supplement for the isolation, enumeration and differentiation of enterococci in foodstuffs.

2 - COMPOSITIONS

TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

KANAMYCIN AESCULIN AZIDE AGAR BASE

Tryptone	20.00 g
Yeast extract	5.00 g
Sodium chloride	5.00 g
Sodium citrate	1.00 g
Aesculin	1.00 g
Ferric ammonium citrate	0.50 g
Sodium azide	0.15 g
Agar	10.00 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

KANAMYCIN SELECTIVE SUPPLEMENT

(VIAL CONTENTS FOR 500 ML OF MEDIUM)

Kanamycin sulphate	10 mg
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3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Kanamycin Aesculin Azide Agar is prepared according to the formulation described by Mossel *et al.*^{1,2} It is a bile-free selective medium for the isolation, enumeration and differentiation of enterococci in foodstuffs. The medium utilises the selective inhibitory components sodium azide and kanamycin: growth of the majority of unwanted organisms is suppressed. The medium contains the indicator system aesculin and ferrous iron: enterococci hydrolyse aesculin producing black zones around the colonies due to the formation of black iron phenolic compounds derived from the aglucon.³ Essential growth factors are provided by tryptone which is a source of nitrogen, carbon and minerals whereas yeast extract provides vitamins, particularly of the B-group. Sodium chloride is a source of electrolytes and maintains the osmotic equilibrium.

4- DIRECTIONS FOR MEDIA PREPARATION

Suspend 21.3 g of dehydrated medium in 500 mL of cold purified water. Heat to boiling with frequent agitation and sterilize by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and add the content of one vial of Kanamycin Selective Supplement (REF 4240055) reconstituted with 5 mL of a sterile purified water, under aseptic conditions. Mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Kanamycin Aesculin Azide Agar Base

Dehydrated medium appearance	beige, fine, free-flowing powder
Solution and prepared plates appearance	tan with trace blue cast, limpid
Final pH at 20-25 °C	7.0 ± 0.2

Kanamycin Selective Supplement

Freeze-dried supplement appearance	short, dense, white pellet
Reconstituted supplement appearance	colourless, limpid solution

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Kanamycin Aesculin Azide Agar Base	Dehydrated medium	4015522	500 g (11.7 L)
Kanamycin Selective Supplement	Freeze-dried supplement	4240055	10 vials, each for 500 mL of medium

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, incubator and laboratory equipment as required, sterile loops and pipettes, Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents.

8 - SPECIMENS

Food, feed, food chain samples. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards.

9 - TEST PROCEDURE

1. Prepare tenfold dilutions of sample with a suitable diluent.
2. Within 3 hours from the sample preparation, spread 0.1 mL of the inoculum onto the plates.
3. Incubate at 35°C or at 42°C for 18-24 hours (the higher incubation temperature increases the selectivity of the medium).

10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies. Typical enterococci colonies are round, grey, about 2 mm in diameter surrounded by a brown-black zone.



11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T° / T / ATM	EXPECTED RESULTS
<i>E. faecium</i> ATCC 19434	37°C / 24 H / A	grey colonies with black halo
<i>B. subtilis</i> ATCC 6633	37°C / 24 H / A	inhibited

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12- PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Kanamycin Aesculin Azide Agar Base supplemented with Kanamycin Selective Supplement is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

The productivity characteristics are tested by semi-quantitative ecometric technique with the following target strains: *E. faecalis* ATCC 19433, *E. faecium* ATCC 19434, *E. hirae* ATCC 8043, *E. gallinarum* ATCC 49573. The amount of growth and colonies characteristics are evaluated after incubation at 35-37°C for 24 hours: the target strains exhibit a good growth with grey colonies with black halo.

The selectivity is evaluated with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of the non-target strains *S. lactis* ATCC 8780, *A. viridans* ATCC 10400, *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633. After incubation the growth of non-target strains is inhibited.

13 - LIMITATIONS OF THE METHOD

- The identification of isolated strains must be confirmed by suitable tests.

14 - PRECAUTIONS AND WARNINGS

- The medium base and the supplement are for microbiological control only, and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplement shall be used in association according to the described directions.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. Kanamycin Selective Supplement is classified as hazardous. Before use, consult the Material Safety Data Sheets.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- The selective supplement is sterilised by membrane filtration.
- Be careful when opening the metal ring of the supplements vials to avoid injury.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplements as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our products for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium

Upon receipt, store at +10°C / +30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).

Selective supplement

Upon receipt, store the product in the original package at +2°C / +8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilized product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/tubes/bottles) and the applied storage conditions (temperature and packaging). According to Baird RM *et al* the self-prepared plates can be stored at +2°C / +8°C for 7 days.³













16 – REFERENCES

- Mossel DAA, Bijker PGH, Eeldering J.: Streptokokken der Lancefield-Gruppe D in Lebensmitteln und Trinkwasser - Ihre Bedeutung, Erfassung und Bekämpfung. - Arch. f. Lebensmittelhyg., 1978; 29: 121-127
- Mossel DAA, Bijker PGH, Eeldering J. VanSpreekens KA. In: Streptococci, edited by Skinner LH and Quesnel LB. SAB Symposium Series n° 7. Academic Press, London.
- Baird RM, Corry JEL, Curtis GDW. Pharmacopoeia of Culture Media for Food Microbiology. Proceedings of the 4th International Symposium on Quality Assurance and Quality Control of Microbiological Culture Media, Manchester 4-5 September, 1986. Int J Food Microbiol 1987; 5:195-196





TABLE OF APPLICABLE SYMBOLS

 or  Catalogue number	 Batch code	 Manufacturer	 This side up	 Store in a dry place	 Fragile
 Temperature imitation	 Content sufficient for <n> tests	 Consult Instructions for Use	 Use by	 Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2023/02

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

