Instructions for Use

REF - KM0189

Sheep Blood Agar Base

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use. KM0189 Sheep Blood Agar Base a general-purpose medium, formulated to be compatible with defibrinated sheep blood, and is suitable for the isolation of most organisms including many fastidious anaerobes.

2. Composition*

Ingredient	g/L
Tryptone	14.0
Peptone	4.5
Yeast extract	4.5
Sodium chloride	5.0
Agar	12.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Alternative blood-based culture mediums can result in mixed haemolytic reactions for some *Streptococcus* species. This is thought to occur due to the trace amounts of fermentable carbohydrates in yeast extract and the physiological difference between sheep and horse blood [(1)].

Sheep Blood Agar Base has been developed to provide a nutrient rich medium compatible with sheep blood for the cultivation of many bacteria, especially fastidious *Streptococcus* species, without affecting haemolytic reactions.

4. Principle

The tryptone, peptone, and yeast extract provide the required nitrogen, carbon and vitamins in the medium. Sodium chloride maintains the osmotic balance.

5. Preparation Instructions

Suspend 40.0g/L of dehydrated culture medium in 1 litre of deionised/purified water and allow to soak whilst mixing for 10 minutes.
Heat with frequent agitation and boil for one minute to completely dissolve powder.

Autoclave to sterilise at 121°C for 15 minutes before cooling in a water bath to 45-50°C. Once cooled add 5-10% of defibrinated sheep blood.

Gently homogenize the final medium before aseptically dispensing the specified volume into appropriate sterile containers and allowing to cool.

Prepared media may be kept at between 2 and 8°C for up to 14 days away from direct sunlight.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Straw fine powder	Red firm gel
pH	N/A	7.3 ± 0.2

7. Materials Provided

KM0189 can be provided in the formats detailed below. Each tub is labelled with product name, product code, lot number, expiry date, instructions, and appropriate warnings.

Product Code	Product Format
KM0189-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0189-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0189-10KG-10000	1 x 10kg Dehydrated Culture Media Tub

8. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., autoclave, sterile loops or swabs, collection containers, incubators, and quality control organisms.

9. Specimens

Sheep Blood Agar is suitable for the testing of the following specimens:

- Samples for the testing and isolation of most organisms including fastidious anaerobes could include swabs from the infected areas, biopsy material, pus, bone, blood, and urine.

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

10. Test Procedures and Interpretation of results

Allow plates to come to room temperature.

Based on sample type and information provided, check to see if the specimen needs to be pre-enriched prior to inoculation to the prepared medium.

Inoculate media directly with the sample, or subculture onto media after incubation in enrichment broth where required.

Clinical Specimens:

Inoculate specimen, or pre-enriched sample, directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker. Incubate 37 ± 1°C aerobically for 24-48 hours.

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). Perform further biochemical, serological, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
B. fragilis (NCTC 9343)	37 ± 1°C aerobically for 24-48 hours	Growth: Grey colonies
E. coli (NCTC 12241)	37 ± 1°C aerobically for 18-24 hours	Growth: Opaque/grey colonies
S. pyogenes (NCTC 12696)	37 ± 1°C aerobically for 18-24 hours	Growth: White colonies with β-haemolysis
S. pneumoniae (ATCC 6305)	37 ± 1°C in 5% CO_2 for 18-24 hours	Growth: Grey/green colonies with α-haemolysis
S. aureus (NCTC 12981)	37 ± 1°C aerobically for 18-24 hours	Growth: White colonies

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium, and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

12. Performance

To fully verify KM0189 Sheep Blood Agar Base performance, dehydrated culture media samples were used to prepare Blood Agar Base with 5% Sheep Blood and tested to assess colony morphology and recovery level (where an acceptable range is ≥70% and ≤120%) compared to a non-selective reference medium. Prepared samples were inoculated with 30-150cfu and incubated at 37 ± 1°C in the atmosphere indicated in the Quality Control table above for 18-48 hours. All samples of prepared media produced grew and showed good recovery and the correct morphology of the required test organisms: *Bacteroides fragilis* (NCTC 9343), *Escherichia coli* (NCTC 12241), *Streptococcus pyogenes* (NCTC 12696), *Streptococcus pneumoniae* (ATCC 6305) and *Staphylococcus aureus* (NCTC 12981). Therefore, it can be concluded that KM0189 Sheep Blood Agar Base, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- Haemolytic reactions of beta-haemolytic streptococci may vary depending on incubation atmosphere.
- Due to natural variation, some strains may grow poorly on this medium.

14. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to KM0189 Material Safety Data Sheet.

15. Storage conditions and Shelf life

Store product in the original container with the lid tightly closed at between 10 and 30°C in low humidity conditions away from direct sunlight. Kept under these conditions, the product may be used up to the date of expiry shown on the product label.

Do not use if the product is not free-flowing or displays any sign of colour change, formation of large lumps or hardening of the powder. Additionally, do not use medium if it has been stored inappropriately, the packaging has been damaged or has passed the expiry date.

Dehydrated culture media does not need to be used all at once; replace the cap and ensure that the container is tightly closed and stored according to labelled instructions.
Dispose of in accordance with local and national authority requirements.

16. References

1. Spector, W. S. (1956). Handbook of biological data. Handbook of biological data. Philadelphia: W. B. Saunders Company.

Version History*

001 09/10/23 - New Document Created

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

EC REP

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TABLE OF APPLICABLE SYMBOLS

REF o REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place