

Instructions for Use

REF - KM0025

Tryptone Soya Broth (TSB)

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use. KM0025 Tryptone Soya Broth (TSB) is a nutritious medium for the non-selective enrichment of non-fastidious and some fastidious organisms ⁽¹⁾.

2. Composition*

<u>Ingredient</u>	<u>g/L</u>
Tryptone	17.0
Soya peptone	3.0
Sodium chloride	5.0
Glucose	2.5
Di potassium hydrogen phosphate	2.5

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

The use of Tryptone Soya Broth (TSB) and modifications of the medium are described in ISO10273 ⁽²⁾ and the medium, with the addition of glycerol, can also be used in the process of preserving positive strains as frozen stock cultures at an early stage of confirmation. Tryptone Soya Broth also conforms to the requirements of the Harmonised USP/EP/JP ^(3 4 5). TSB is recommended by ISO11133 ⁽⁶⁾ for the preparation of working cultures.

4. Principle

Casein peptone and soya peptone provide nitrogen, vitamins, and minerals. The natural sugars from soya peptone promote bacterial growth. Sodium chloride is for the osmotic balance. Glucose is present to supply energy, and dipotassium hydrogen phosphate is a buffering agent to help maintain pH.

5. Preparation Instructions

Suspend 30.0 of dehydrated culture medium in 1 litre of deionised/purified water and allow to soak whilst mixing for 10 minutes.

Dispense into appropriate containers and autoclave to sterilise at 121°C for 15 minutes.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Pale straw fine powder	Pale straw liquid
pH	N/A	pH = 7.3 ± 0.2

7. Materials Provided

KM0025 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
KM0025-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0025-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0025-10KG-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

KM0025 Tryptone Soya Broth is suitable for the testing of the following specimens:

- Clinical specimens: Tryptone Soya Broth is suitable for use in the suspension, enrichment and cultivation of microbial strains isolated from other culture media and for the preparation of inoculum in quality control test procedures.
- Pharmaceutical specimens: Tryptone Soya Broth is used on products on which the sterility test or the detection of specific microorganism is required.

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

Aerobic Cultivation:

Tryptone Soya Broth may be used for the cultivation of aerobes and facultative anaerobes, including some fungi. Cultures should be examined at frequent intervals, as maximum growth is reached earlier than with less nutritious media and the phase of decline consequently begins sooner.

Anaerobic Cultivation:

The addition of a small amount of agar renders the broth suitable for the cultivation of obligate anaerobes, such as *Clostridium* spp. For this purpose, the broth (with added agar) should be used soon after sterilisation or heated and cooled just before inoculation.

Blood Culture:

The superior growth-promoting properties of Tryptone Soya Broth make it especially useful for the isolation of organisms from blood or other normally sterile body fluids. Anticoagulants such sodium citrate may be added to the broth prior to sterilisation.

After incubation, examine broths for turbidity; examine subcultures on agar for colonies (typical colony appearance outlined in Quality Control table below). Perform further biochemical or mass spectroscopy testing to confirm identity of isolated from subcultures. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
<i>S. aureus</i> (NCTC 10788)	30-35°C for 18-24 hours	Growth: Turbid. Subplate: White colonies
<i>E. coli</i> (NCTC 12923)	30-35°C for 18-24 hours	Growth: Turbid. Subplate: Grey colonies
<i>B. subtilis</i> (NCTC 10400)	30-35°C for 18-24 hours	Growth: Turbid. Subplate: Cream colonies
<i>P. aeruginosa</i> (NCTC 12924)	30-35°C for 18-24 hours	Growth: Turbid. Subplate: Green/grey colonies
<i>C. albicans</i> (NCPF 3179)	20-25°C for 48 hours	Growth: Turbid. Subplate: White colonies
<i>A. brasiliensis</i> (NCPF 2275)	20-25°C for 72 hours	Growth: Turbid. Subplate: White colonies, reverse side pale yellow

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 KM0025 Tryptone Soya Broth (TSB) performance, dehydrated culture media samples were used to prepare Tryptone Soya Broth (TSB) and tested to assess turbidity, colony morphology and recovery level when subcultured onto E&O PP0280 Tryptone Soya Agar (TSA) (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*) and E&O PP0220 Sabouraud Dextrose Agar (SDA) with Chloramphenicol (*Candida albicans* (NCPF 3179) and *Aspergillus brasiliensis*). Prepared samples were inoculated with 30-150cfu and incubated at the appropriate temperatures and times as outlined in the Quality Control table above. All samples of prepared media showed expected recovery and morphology of the required test organisms: *S. aureus* (NCTC 10788), *E. coli* (NCTC 12923), *B. subtilis* (NCTC 10400), *P. aeruginosa* (NCTC 12924), *C. albicans* (NCPF 3179) and *A. brasiliensis* (NCPF 2275). Therefore, it can be concluded that KM0025 Tryptone Soya Broth (TSB), meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- KM0025 is not suitable for the cultivation of fastidious organisms such as *Neisseria* or *Haemophilus* species, or for the growth of strict anaerobes.
- Sub-culture onto suitable solid culture media is necessary for purification of the culture and to perform identification tests.
- 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 KM0025 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. Mac Faddin, J. (1985) Media for Isolation-cultivation-identification-maintenance of Medical Bacteria. 7th ed. Baltimore: Williams and Wilkins.
2. ISO (2017) 10273:2017 Microbiology of the food chain - Horizontal method for the detection of pathogenic *Yersinia enterocolitica*. Geneva: ISO.
3. United States Pharmacopeial Convention (2019) The United States Pharmacopeia USP42, The National Formulary NF37. Rockville, MD: United States Pharmacopeial Convention.
4. Council of Europe (2019) European Pharmacopoeia. 10th ed., Strasbourg: Council of Europe.
5. The Ministry of Health, Labour and Welfare (2021) The Japanese Pharmacopoeia. 18th ed. Tokyo: Yakuji Nippo Limited.
6. ISO (2014) 11133:2014 Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media. Geneva: ISO.

Version History*

001 14/04/23 - New Document Created

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



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IFU/KM0025 REV. 001

TABLE OF APPLICABLE SYMBOLS

 REF Catalogue number	 LOT Batch code	 IVD <i>In vitro</i> 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