

Instructions for Use

Tryptone Soya Agar (USP)

Bottled Media

REF - BM1432

1. Intended Use

BM1432 Tryptone Soya Agar (USP) is a general-purpose, non-selective medium capable of supporting the growth of a wide range of aerobic and anaerobic micro-organisms.

2. Composition*

<u>Ingredient</u>	<u>g/L</u>
Pancreatic digest of casein	15.0
Papaic digest of soybean	5.0
Sodium chloride	5.0
Agar	15.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Tryptone Soya Agar, also known widely as TSA, is used for a wide range of applications, including culture storage, enumeration of cells, isolation of pure cultures, or simply general culture. It has been found to be useful in cosmetic testing, water, and wastewater applications. Tryptone Soya Agar may be used in sterility testing and environmental monitoring within pharmaceutical cleanrooms and sterile facilities ⁽¹⁾. This medium meets the requirements of the Harmonized USP/EP/JP ^(1 2 3) and is based on the original formulation described by Leavitt *et al.* in 1955 ⁽⁴⁾. Tryptone Soya Agar is recommended as a reference medium when testing selective media, to measure the degree of inhibition ⁽⁵⁾. In environmental monitoring applications, it is common for plates to be incubated at 30-35°C for bacterial colonies and 20-25°C for mould and fungi.

4. Principle

Casein peptone and Soya peptone provide nitrogen, carbon, and other essential nutrients. Sodium chloride provides osmotic balance. Tryptone Soya Agar (USP) will support the growth of both aerobic and anaerobic organisms depending on incubation conditions.

5. Physical Characteristics

	<u>Appearance and Colour</u>	<u>pH</u>
Medium	Straw firm gel	7.3 ± 0.2

6. Materials Provided

BM1432 Tryptone Soya Agar (USP) can be provided in the formats detailed below. Each bottle is ink-jet printed or labelled with (abbreviated) product name, product code, lot number and expiry date.

<u>Product Code</u>	<u>Product Format</u>
BM1432-J300-250	6 x 250ml in 300ml Sirop Bottles
BM1432-J500-400	6 x 400ml in 500ml Sirop Bottles
BM1432-J500-500	6 x 500ml in 500ml Sirop Bottles
BM1432-M002-15	50 x 15ml in Thin Wall Universals
BM1432-R300-200	6 x 200ml in 300ml Sirop Bottles

7. Materials Needed but not Provided

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

8. Specimens

BM1432 Tryptone Soya Agar (USP) is suitable for the testing of the following specimens:

- Cosmetics
- Food and Environmental: products for human consumption, animal feed and environmental samples
- Waters and wastewater

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.

9. Test Procedures and Interpretation of results

Follow local, national, or international guidelines.

Based on sample type and information provided, determine whether the specimen requires pre-enrichment prior to inoculation to the prepared medium.

Food/Water/Environmental Specimens

- Process the food/water specimen as required by local guidelines.
- Inoculate an isolated pure culture directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker.

Cosmetic Specimens

- Process the cosmetic specimen as required by local guidelines.
- For liquid/aerosol samples, prepare a homogenised suspension using a ratio of 1:9 of sample to diluent such as E&O BM4284 Modified Lethen Broth.
- For solid/powder samples, prepare a homogenised suspension using a ratio of 1:1:8 of sample to Tween 80 and diluent such as E&O BM4284 Modified Lethen Broth.
- For cream/oil-based samples, prepare a homogenised suspension using a ratio of 1:1:8 of sample to Tween 80 and diluent such as E&O BM4284 Modified Lethen Broth, ensuring beads are added to guarantee emulsification.
- Inoculate homogenised specimens, and dilutions thereof, directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker.

Incubate the plates at 30-35°C for between 18 hours and 5 days depending on the microorganism required ^(1 2 3).

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

10. Quality Control

Organism	Incubation	Result (Specificity)
<i>P. aeruginosa</i> (ATCC 9027)	30-35°C for 18-24 hours	Growth: Cream colonies
<i>C. albicans</i> (ATCC 10231)	20-25°C for 48 hours	Growth: White colonies
<i>S. aureus</i> (ATCC 6538)	30-35°C for 18-24 hours	Growth: Yellow colonies
<i>B. subtilis</i> (ATCC 6633)	30-35°C for 18-24 hours	Growth: Cream colonies
<i>A. brasiliensis</i> (NCPF 2275)	20-25°C for 72 hours	Growth: White colonies with pale yellow underside and hyphae
<i>E. coli</i> (ATCC 8739)	30-35°C for 18-24 hours	Growth: Cream 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).

11. Performance

To fully verify performance of BM1432 Tryptone Soya Agar (USP), samples were tested to assess colony morphology and recovery when incubated at the temperature and times outlined in the Quality Control table above. An acceptable recovery range is ≥70% and ≤120% compared to a non-selective reference medium. All samples grew and showed good recovery and the correct morphology of the required test organisms: *Pseudomonas aeruginosa* (ATCC 9027), *Candida albicans* (ATCC 10231), *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Aspergillus brasiliensis* (NCPF 2275) and *Escherichia coli* (ATCC 8739). Therefore, it can be concluded that BM1432 Tryptone Soya Agar (USP), meets performance criteria when used according to the instructions outlined above.

12. Limitations of the Medium

- Biochemical, serological, molecular, or mass spectrometry testing using pure cultures may be necessary for complete identification.
- Due to natural variation, some strains may grow poorly on this medium.

13. 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 E&O BM1432 Bottled Media Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store BM1432 in the original bottle with the lid tightly closed at between 15 and 30°C. Kept under these conditions it may be used up to date of expiry shown on product label.

Dispose of in accordance with local and national authority requirements. Do not use medium if it is contaminated, discoloured or out of date. Additionally, do not use medium if it has been stored inappropriately or the packaging has been damaged.

15. References

1. Council of Europe (2019) European Pharmacopoeia. 10th ed., Strasbourg: Council of Europe.
2. The Ministry of Health, Labour and Welfare (2021) The Japanese Pharmacopoeia. 18th ed. Tokyo: Yakuji Nippo Limited.
3. United States Pharmacopeial Convention (2019) The United States Pharmacopeia USP42, The National Formulary NF37. Rockville, MD: United States Pharmacopeial Convention.
4. Leavitt, J., Naidorf, I. and Shugaevsky, P. (1955) Aerobes and anaerobes in endodontics. Part I. The undetected anaerobes in endodontics. Part II. Sensitive culture medium for the detection of both aerobes and anaerobes. NYSDJ, 25, pp.377-382.
5. Orth, D. (1993) Handbook of Cosmetic Microbiology. New York: Marcel Dekker.

Version History*

001 14/11/23 - New Document Created

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



E&O Laboratories Ltd, Burnhouse, Bonnybridge, Scotland, FK4 2HH, Tel: +44 (0) 1324 840 404, info@eolabs.com, www.eolabs.com



Syntec Scientific Ltd., Unit 2 The Business Centre, Northwest logistics Park, Ballycoolin, Dublin 15, Ireland D1SPY00

TABLE OF APPLICABLE SYMBOLS

IFU/BM1432 REV. 001

 REF Catalogue number	 LOT Batch code		 Manufacturer	 Use by
 Temperature limitation	 Contents sufficient for <n> tests	 Consult Instructions for Use	 Keep away from direct light	 Store in a dry place