Tributyrin Agar Base

Without Tributyrin · Catalogue No. AS-1453

1. Product Identification

Product name	Tributyrin Agar Base (without Tributyrin)
Catalogue No.	AS-1453
Product type	Dehydrated base medium
Manufacturer	Ausamics Pty Ltd, Epping, VIC, Australia
Pack size	500 g

2. Intended Use

Base for Tributyrin Agar, used for the detection and enumeration of lipolytic microorganisms in food and other material, including staphylococci, pseudomonads, clostridia and marine flavobacteria.

This product is the base medium only. Tributyrin (glyceryl tributyrate) is not included and must be added by the user at 10 mL per litre and emulsified before sterilisation.

3. Mode of Action

Special peptone and yeast extract supply nitrogen, amino acids, vitamins and trace elements. Tributyrin, emulsified into the molten medium by the user, disperses as fine droplets and renders the agar opaque. Extracellular lipases and esterases hydrolyse tributyrin to butyric acid and glycerol, clearing the emulsion around the colony and producing a transparent halo.

4. Formulation of the Base (g/L)

Ingredient	g/L
Special Peptone	5.0
Yeast Extract	3.0
Agar	12.0
Total	20.0

Formula may be adjusted to meet performance criteria.

5. Supplement (not included)

Supplement	CAS No.	Amount	Function
Tributyrin (glyceryl tributyrate)	60-01-5	10 mL/L	Lipid substrate; forms the opaque emulsion that lipolysis clears

6. Preparation

1. Suspend 20 g of base in 990 mL of purified water.
2. Add 10 mL of tributyrin (not included).
3. Heat to boiling with constant agitation until the agar is completely dissolved.
4. Emulsify thoroughly — homogenise or mix vigorously until the medium is uniformly opaque.

5. Sterilise by autoclaving at 121 °C for 15 minutes.
6. Cool to 45–50 °C, mix well to re-emulsify, and pour into sterile glass Petri dishes.

Alternative order: sterilise the base alone at 121 °C for 15 minutes, cool to about 80 °C, then add the tributyrin and emulsify immediately. Emulsifying before sterilisation generally gives a more uniform and more stable emulsion.

Pour into glass Petri dishes. Tributyrin can attack polystyrene, and plastic dishes may craze or cloud, which makes zones difficult to read.

7. Incubation & Reading

Application	Temperature	Time	Notes
Food and dairy organisms	30–37 °C	24–48 h	Read clear zones against the opaque background
Slow lipolysis	30–37 °C	up to 5 days	Hold negative plates before reporting a negative result
Marine flavobacteria, psychrotrophs	20–25 °C	3–5 days	Lower temperature for marine and chill-tolerant organisms

8. Interpretation

Positive	Clear or translucent zone around the colony — tributyrin hydrolysed
Negative	Medium remains uniformly opaque; growth without clearing
Uneven opacity	Broken or incomplete emulsion; do not read the plate

9. Quality Control (complete medium)

Organism	Strain	Growth	Lipolysis
Staphylococcus aureus subsp. aureus	ATCC 25923	Luxuriant	Clear zone (lipolysis positive)
Bacillus subtilis subsp. spizizenii	ATCC 6633	Luxuriant	Clear zone (lipolysis positive)
Clostridium sporogenes	ATCC 11437	Luxuriant	Clear zone (lipolysis positive)
Pseudomonas aeruginosa	ATCC 27853	Luxuriant	Clear zone (lipolysis positive)
Clostridium perfringens	ATCC 12924	Luxuriant	No clear zone (negative)
Escherichia coli	ATCC 25922	Luxuriant	No clear zone (negative)

Cultural response read after 24–48 h at 35–37 °C on medium containing 10 mL/L tributyrin.

10. Storage & Shelf Life

Dehydrated base: 15–25 °C, dry, tightly closed, protected from light. The powder is hygroscopic. Prepared plates: store below 8 °C and use within 4 weeks.

11. Limitations

- Without added tributyrin the medium is a plain nutrient agar and is not differential. The base alone cannot detect lipolysis.

- Tributyrin is a short-chain triglyceride. Organisms that hydrolyse only long-chain fats may give a weak reaction or none, and the medium detects esterase activity as well as true lipase.
- The quality of the emulsion decides how readable the plate is. Poor emulsification gives uneven opacity that can be mistaken for zones of clearing.
- The medium is not selective. Accompanying flora grow alongside lipolytic organisms.
- A clear zone shows hydrolysis of tributyrin only. It does not identify the organism; confirm by biochemical or molecular methods.
- Zone diameter depends on inoculum size, agar depth and incubation time. Compare only plates poured to the same depth in the same batch.

12. Literature / References

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