

Tributyryn Agar, Base

Medium proposed by ANDERSON (1939) for the detection and enumeration of lipolytic microorganisms in foodstuffs and other materials. The medium can also be used for the detection of lipase in various bacterial species such as staphylococci (INNES 1956), clostridia (WILLIS 1960), *Pseudomonas*, marine flavobacteria (HAYES 1963) etc.

Mode of Action

The culture medium contains tributyrin as a reactant; degradation of this compound gives rise to clear zones surrounding the lipolytic colonies in the otherwise turbid culture medium.

Typical Composition (g/litre)

Peptone from meat 2.5; peptone from casein 2.5; yeast extract 3.0; agar-agar 12.0.

Also to be added:

Tributyryn 10.0 ml.

Preparation

Suspend 20 g/litre, add 10 ml neutral tributyrin/litre, mix uniformly and autoclave (15 min at 121 °C). While shaking frequently (emulsification of the tributyrin) cool to at least 50°C (stabilization of the emulsion) and pour plates. Allow the plates to solidify rapidly.

pH: 7.5 ± 0.2 at 25 °C.

The plates are turbid and yellowish.

- **The culture medium must contain a uniformly turbid emulsion. If the emulsion separates, the effectiveness of the culture medium is affected.**

EL SADEK and RICHARDS (1957) reported that other glycerides such as triolein and trilinolein can be used instead of tributyrin. According to RAPP (1978), better emulsification of tributyrin can be achieved if 4 ml polyoxyethylene-(20)-hydrated ricinus oil is added to 1 litre of the culture medium.

Experimental Procedure and Evaluation

Inoculate the culture medium by the pour-plate method or by spreading the sample material on the surface of the plates.

Incubation: up to 72 hours under optimal conditions (e.g. 28 °C).

Lipolytic microorganisms produce colonies which are surrounded by clear zones in the otherwise turbid culture medium.

Quality control

Test strains	Growth	Clear zones
<i>Escherichia coli</i> ATCC 25922	good / very good	-
<i>Salmonella typhimurium</i> ATCC 14028	good / very good	-
<i>Pseudomonas aeruginosa</i> ATCC 27853	good / very good	+
<i>Staphylococcus aureus</i> ATCC 25923	good / very good	+
<i>Bacillus subtilis</i> ATCC 6633	good / very good	+
<i>Penicillium commune</i> ATCC 10428	poor / fair	+

Literature

ANDERSON, J.A.: The use of tributyrin agar in dairy bacteriology. - *Ber. 3. Int. Mikrobiol. Kongress*, **3**; 726-728 (1939)

EL SADEK, G.M., a. RICHARDS, T.: Nile blue, aniline blue and neutral red as indicators of lipolysis. - *J. Appl. Bact.*, **20**; 137 (1959).

INNES, A.G.: Coagulase positive Staphylococci from bulk milk supplies low in solids-notfat. - *J. Appl. Bact.*, **19**; 39-45 (1956).

HAYES, P.R.: Studies on marine flavobacteria. - *J. Gen. Microbiol.*, **30**; 1-19 (1963).

RAPP, M.: Elektive Nährmedien zum Nachweis von Lipolyten. - *Milchwirtschaft.*, **33**; 493-496 (1978).

WILLIS, A.T.: The lipolytic activity of some clostridia. - *J. Path. Bact.*, **80**; 379-390 (1960).

Ordering Information

Product	Merck Cat. No.	Pack size
Tributyryn Agar, Base	1.01957.0500	500 g
Glycerol tributyrate (Tributyryn)	1.01958.0100	100 ml

Manufacturer	Product
ICI Chemicals, Essen, BRD	Polyoxyethylene-(20)-hydrated Ricinus oil