EPA

Fluorocult® LMX Broth modified

Enrichment for the simultaneous detection of total coliforms and E.coli in water, food and dairy products by the fluorogenic procedure.

Mode of Action

LMX Broth first described by MANAFI and KNEIFEL (1989) was modified by MANAFI and OSSMER (1993) to improve the substrate utilization, to increase sensitivity and at the same time reduce the overall incubation time to 24 hours.

Fluorocult® LMX Broth Modified contains phosphate buffer to guarantee a high growth rate for total coliforms. Lauryl sulfate largely inhibits the accompanying Gram-positive flora. By adding the chromogenic substrate 5-bromo-4-chloro-3-indolyl-ß-D-galactopyranoside, which is cleaved by coliforms and the fluorogenic substrate 4-methylumbelliferyl-ß-D-glucuronide, which is highly specific for E.coli, the simultaneous detection of total coliforms and E.coli is possible. A color change of the broth from yellow to blue-green indicates the presence of coliforms. In addition a blue fluorescence under long-wave UV light permits the rapid detection of E.coli. As tryptophan is added to the broth, the indole reaction is easily done by adding Kocavs reagent. The formation of a red ring additionally confirms the presence of E.coli. The enzyme synthesis is amplified by 1-isopropyl-ß-D-1-thio-galactopyranoside and increases the ß-D-galactosidase activity.

Typical Composition (g/litre)

Tryptose 5.0; sodium chloride 5.0; sorbitol 1.0; tryptophan 1.0; dipotassium hydrogen phosphate 2.7; potassium dihydrogen phosphate 2.0; lauryl sulfate sodium salt 0.1; 5-bromo-4-chloro-3-indolyl-ß-D-galactopyranoside (X-GAL) 0.08; 4-methylumbelliferyl-ß-D-glucuronide (MUG) 0.05; 1-isopropyl-ß-D-1-thio-galactopyranoside (IPTG) 0.1.

Preparation

Food testing:
Suspend 17 g (single strength) in 1 liter of purified water. Heat to boiling to dissolve completely. Fill up to 20 ml aliquots into tubes. Autoclave for 15 min. at 121 °C.

Water testing:
If 100 ml water samples (e.g. drinking water) are to be tested, suspend 34 g (double strength) in 1 liter of purified water. Heat to boiling to dissolve completely. Transfer 100 ml aliquots into bottles (minimum: 250 ml capacity). Autoclave for 15 min. at 121 °C.

pH: 6.8 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish-brown.

Experimental Procedure and Evaluation

Application varies with the method/samples used for water or food testing.

Incubation: 18-24 hours at 36 ± 1.0 °C aerobically. If incubated at room temperature (+20 to +25 °C) the incubation time is prolonged to 48 hours.

Total coliforms: broth color has changed to blue-green.

E.coli: blue-green color of the broth and blue fluorescence using long-wave UV light source (366 nm). Overlay with Kovacs reagent for the indole reaction - a red ring additionally confirms the presence of E.coli.

Literature

HAHN, G., a. WITTROCK, E.: Comparison of chromogenic and fluorogenic substances for differentiation of Coliforms and Escherichia coli in soft cheeses. - Acta Microbiologic Hungarica 38 (3-4); 265-271 (1991).

MANAFI, M.: Schnellnachweis von Bakterien mittels fluorogener und chromogener Substrate. - Forum Städte-Hygiene 41; 181-184 (1990).

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MANAFI, M., KNEIFEL, W.: Fluorogenic and chromogenic substrates. - A promising tool in Microbiology. - Acta Microbiologica Hungarica 38 (3-4); 293-304 (1991).

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MANAFI, M., KNEIFEL, F., BASCON, S.: Fluorogenic and chromogenic substrates used in bacterial diagnosis. - Microbiol. Rev. 55; 335-348 (1991).

OSSMER, R.: Simultaneous Detection of Total Coliforms and E.coli - Fluorocult LMX-Broth. - 15th International Symposium/FOOD MICRO 1993. The International Committee on Food Microbiology and Hygiene, Bingen/Rhine (1993).

Ordering Information

Product	Ordering No.	Pack size
Fluorocult® LMX Broth modified	1.10620.0500	500 g
Bactident® Indole (dropper bottle)	1.11350.0001	1 x 30 ml
KOVÁCS Indole Reagent	1.09293.0100	100 ml
UV Lamp (366 nm)	1.13203.0001	1 ea

Quality control

Test strains	Colour change to blue-green	Fluorescence	Indole reaction
Escherichia coli ATCC 25922	+	+	+
Klebsiella pneumoniae ATCC 31488	+	-	-
Enterobacter cloacae ATCC 13047	+	-	-
Citrobacter brakii ATCC 6750	+	-	
Citrobacter freundii ATCC 8090	+	-	
Shigella flexneri ATCC 12022	-	-	
Salmonella typhimurium ATCC 14028	-	-	
Aeromonas hydrophila ATCC 7966	-	-	
Pseudomonas aeruginosa ATCC 10145	+	-	-