

TM 1689 – FLUOROGENIC LMX BROTH MODIFIED (as per MANAFI & OSSMER)

INTENDED USE

Selective medium for detection of total coliforms and *E. coli* from foods and water.

PRODUCT SUMMARY AND EXPLANATION

LMX Broth first described by MANAFI and KNEIFEL (1989) was modified by MANAFI and OSSMER (1933) to improve the substrate utilization, to increase sensitivity and at the same time reduce the overall incubation time to 24 hours.

COMPOSITION

Ingredients	Gms / Ltr
Tryptone	5.000
Sodium chloride	5.000
Sorbitol	1.000
Tryptophan	1.000
Dipotassium hydrogen phosphate	2.700
Potassium dihydrogen phosphate	2.000
Lauryl sulphate sodium salt	0.100
5-Bromo-4-chloro-3-indoyl- β -D-galactopyranoside (X-GAL)	0.080
4- Methylum-belliferyl- β -D-glucuronide(MUG)	0.050
1-isopropyl- β -D-1-thio-galactopyranoside(IPTG)	0.100

PRINCIPLE

The medium consists of phosphate buffer to guarantee high growth rate for total coliforms. Lauryl sulphate inhibits gram positive flora. By adding chromogenic substrate 5-Bromo-4-chloro-3-indoyl- β -D-galactopyranoside (X-GAL), which is cleave by coliforms and the fluorogenic substrate 4- Methylum-belliferyl- β -D-glucuronide(MUG), which is highly specific for *E.coli*, the simultaneous detection of total coliforms and *E.coli* is possible. The colour change of the broth from yellow to blue-green indicates the presence of coliforms. In addition 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 Kovac's reagent. The formation of red confirms the presence of *E.coli*. the enzyme synthesis is amplified by 1-isopropyl- β -D-1-thio-galactopyranoside(IPTG) and increases β -D Galactosidase activity.

INSTRUCTION FOR USE

- Dissolve 17.00 grams in 1000 ml purified/distilled water for food samples and 34.00 grams (double strength) in 1000 ml distilled water for water samples.
- Heat to boiling to dissolve the medium completely. Fill up to 20 ml aliquot into tubes.
- Sterilize in autoclave at 15psi pressure (121°C) for 15 minutes.

QUALITY CONTROL SPECIFICATIONS



Appearance of Powder : Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium : Yellowish brown to clear solution.
pH (at 25°C) : 6.8 ± 0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Colour change to blue-green	Fluorescence	Indole reaction	Incubation Temperature	Incubation Period
<i>Klebsiella pneumoniae</i>	13488	50-100	Positive reaction	Negative reaction	Negative reaction	35-37 °C	18 - 24 Hours
<i>Escherichia coli</i>	25922	50-100	Positive reaction	Positive reaction, blue colour	Positive reaction, red ring formation	35-37 °C	18 - 24 Hours
<i>Enterobacter cloacae</i>	13047	50-100	Positive reaction	Negative reaction	Negative reaction	35-37 °C	18 - 24 Hours
<i>Citrobacter brakii</i>	6750	50-100	Positive reaction	Negative reaction	-	35-37 °C	18 - 24 Hours
<i>Citrobacter freundii</i>	8090	50-100	Positive reaction	Negative reaction	-	35-37 °C	18 - 24 Hours
<i>Shigella flexneri</i>	12022	50-100	Negative reaction	Negative reaction	-	35-37 °C	18 - 24 Hours
<i>Salmonella Typhimurium</i>	14028	50-100	Negative reaction	Negative reaction	-	35-37 °C	18 - 24 Hours
<i>Aeromonas hydrophila</i>	7966	50-100	Negative reaction	Negative reaction	-	35-37 °C	18 - 24 Hours
<i>Pseudomonas areuginosa</i>	10145	50-100	Positive reaction	Negative reaction	Negative reaction	35-37 °C	18 - 24 Hours

PACKAGING:

In pack size of 100 gm bottles.

STORAGE



Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.










Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

1. HAHN, G., a. WITTRÖCK, E.: Comparison of chromogenic and fluorogenic substances for differentiation of Coliforms and Escherichia coli in soft cheeses. - Acta Microbiologica Hungarica 38 (3-4); 265-271 (1991).
2. MANAFI, M.: Schnellnachweis von Bakterien mittels fluorogener und chromogener Substrate. - Forum Städte-Hygiene 41; 181-184 (1990).
3. MANAFI, M.: Diagnostik von Mikroorganismen mittels fluorogener und chromogener Substrate. - Ernährung/Nutrition 15; Nr.10 (1991).
4. MANAFI, M., KNEIFEL, W.: Fluorogenic and chromogenic substrates. - A promising tool in Microbiology. - Acta Microbiologica Hungarica 38(3-4); 293-304 (1991).
5. MANAFI, M., KNEIFEL, W.: Ein kombiniertes Chromogen-Fluorogen- Medium zum simultanen Nachweis der Coliformengruppe und von E.coli in Wasser. - Zbl. Hygiene und Umweltmedizin 189; 225-234 (1989)
6. MANAFI, M., KNEIFEL, F., BASCON, S.: Fluorogenic and chromogenic substrates used in bacterial diagnosis. - Microbiol. Rev. 55; 335-348 (1991).
7. OSSMER, R.: Simultaneous Detection of Total Coliforms and E.coli - Fluoro- cult LMX-Broth. - 15th International Symposium/FOODMICRO 1993. The International Committee on Food Microbiology and Hygiene, Bingen/Rhine (1993).

 GMP Good Manufacturing Practices Certified	 Best Before	 QTY. Quantity	 REF Catalogue Number	 Manufacturer
 Temperature Unit	 LOT/ B. NO. Lot / Batch Number	 Consults Instructions for Use	 QR Code	

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**
Revision: 08 Nov., 2019