Instructions For Use

ENGLISH

Chromatic EC-X Gluc Agar

Medium for detection and enumeration of *E. coli* in water and foodstuffs

INTENDED PURPOSE

Selective and differential chromogenic medium for the detection and enumeration of *Escherichia coli* in water and foodstuffs. This medium is not intended for use in the diagnosis of disease or other conditions in humans.

DESCRIPTION

Chromatic EC X-GLUC Agar is a selective and differential chromogenic medium used for the detection and enumeration of *Escherichia coli* in water samples by membrane filtration.
This medium, also known as Chromogenic E.coli Agar, complies with the recommendations of methods in UNICHIM M.U.1185 and APAT CNR IRSA 7030.

TYPICAL FORMULA* (Per Litre of Purified Water)

Tryptone	20.0
Yeast Extract	5.0
Bile Salts No. 3	1.5
Disodium Hydrogen Phosphate	5.0
Potassium Dihydrogen Phosphate	1.5
Sodium Chloride	5.0
X-Glucuronide	0.06
Tryptophan	1.0
Agar	15.0
Final pH 7.0 ± 0.2 at 25°C	

*Adjusted and/or supplemented as required to meet performance specifications.

METHOD PRINCIPLE

Tryptone provides amino acids, nitrogen, carbon, vitamins and minerals for organism's growth. Yeast extract is a source of vitamins, particularly of B-group. Bile salts inhibit Gram-positive bacteria. Phosphates act as buffer. Sodium chloride maintains the osmotic balance of the medium. X-Glucuronide (5-bromo-4-chloro-3-indoxyl-βD-glucuronide) is the chromogenic substrate which is cleaved by the β-D-glucuronidase enzyme characteristic of *E. coli*. Tryptophan is incorporated into the medium to make possible performing indole test for confirmation of *E. coli*. Agar is the solidifying agent.

PREPARATION

Dehydrated medium

Suspend 54.0 g of the powder in 1 liter of distilled or deionized water. Heat to boil until completely dissolved. Autoclave at 121°C for 15 minutes. Mix well and pour into sterile final containers.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as: Autoclave, sterile Petri plates, test tubes, inoculating loops, incubator, quality control organisms.

TEST PROCEDURE

Ensure there is no visible moisture on the plates before use.

For the examination of water, filter 100 ml of the sample trough a filter membrane (0.45 µm pore diameter), and transfer this onto the surface of the medium.

Incubate aerobically at 44 ± 0.5°C for 18-24 hours.

Alternatively, samples can be inoculated by spread plating, pour plating or by direct streaking on the medium surface.

For more details, consult appropriate guidance.

INTERPRETING RESULTS

Count as *Escherichia coli* all the blue or blue-green colonies showing a positive indole reaction. To evaluate the indole production, transfer some drops of Kovac's Reagent directly onto the colony and observe the formation of a red color. Express results as CFU per ml, allowing for the dilution factor.
β-glucuronidase-negative bacteria, including some *E. coli* strains such as *E. coli* O157, cultivate with colorless colonies on this medium.

STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed.

Store prepared plates at 2-8°C away from light in their original pack until just prior to use. Avoid quick temperature shifts of plated medium to prevent condensation.

Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

SHELF LIFE

Dehydrated medium: 2 years.
Ready-to-use plates: 4 months.

QUALITY CONTROL

Appearance of dehydrated medium: Free-flowing, homogeneous, beige.

Appearance of prepared medium: Light amber, slightly opalescent.

Expected Cultural Response:

Control strain	Inoculum	Incubation	Criteria	Specification
Escherichia coli WDCM 00013 (ATCC® 25922; NCTC 12241)	50-100 CFU	18-24 h / 44 ± 0.5°C	Good growth ($P_R \geq 0.5$)	Blue-green colonies, indole positive
Enterococcus faecalis WDCM 00009 (ATCC® 19433; NCTC 775)	10^4-10^6 CFU		Inhibition	---
Salmonella typhimurium WDCM 00031 (ATCC® 14028; NCTC 12023)	10^3-10^4 CFU		Growth	Colourless colonies, indole negative

A productivity ratio (P_R) of 0.5 is equivalent to a recovery rate of 50%.

Please refer to the actual batch related Certificate of Analysis (CoA).

PERFORMANCE CHARACTERISTICS

Performance testing of Chromatic EC X-GLUC Agar was carried out using the QC strains listed above. The results obtained met the established criteria.

LIMITATIONS

Invalid results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology. The operator should understand the principles of the procedures, including its performance limitations, in advance of operation to avoid potential mistakes.
Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

WARNING AND PRECAUTIONS

For professional use only. Operators must be trained and have certain experience. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

ORDER INFORMATION

Product	Format	Packaging	Ref.
Chromatic EC X-Gluc Agar	Plate 60 mm	20 plates, in blister of 2 pieces (double wrapped)	163722
	Dehydrated medium	100 g	620602
		500 g	610602

Revision History

Revision	Release Date	Change Summary
0	2024-07-08	Updated layout and content, version reset to revision 0

This IFU document and the SDS are available from the online Support Center:

liofilchem.com/ifu-sds

References

1. EN ISO 11133:2014+Amd1:2018. Microbiology of food, animal feed and water -- Preparation, production, storage and performance testing of culture media.
2. ISO 9308-1:2014 Water quality – Enumeration of Escherichia coli and coliform bacteria – Part 1: Membrane filtration method for waters with low bacterial background flora.
3. APAT CNR IRSA Manuali e Linee Guida 2003. Metodi analitici per le acque: Metodo 7030 D Escherichia coli.
4. Bonadonna L. 2001 Escherichia coli nelle acque significato sanitario e metodologie di analisi. ISSN:1125-2464.
5. Metodo UNICHIM 1185 2000 – Acque destinate al consumo umano – Metodo rapido per la ricerca ed enumerazione di Escherichia coli.
6. Clesceri L.S., A.E. Greenberg and A.D. Eaton 1998 Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
7. Delisle G.J., A. Ley 1989 Rapid detection of Escherichia coli in urine samples by a new chromogenic betaglucuronidase assay. J Clin Microbiol 27:778-9.

Table of Symbols

LOT	Batch code
REF	Catalogue number
	Manufacturer
	Use by
	Fragile, handle with care
	Temperature limitation
	Contains sufficient for <n> tests
	Consult instructions for use
	Do not reuse
	Keep away from sunlight

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