Harlequin® Chromogenic Coliform Agar (NCM1005)

Intended Use

Chromogenic Coliform Agar (CCA) is for the enumeration of *Escherichia coli* and Coliforms in water and is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Chromogenic Coliform Agar (CCA) is recommended by ISO 9308-1:2014 for the simultaneous detection and enumeration of β-glucuronidase-positive *Escherichia coli* and β-D-galactosidase positive coliform bacteria from water samples using membrane filtration. Peptone and yeast extract provide a source of nitrogen and essential vitamins. Sodium chloride maintains the osmotic balance and the phosphates buffer the media. Sodium Pyruvate, Sorbitol and Tryptophan aid in the recovery and detection of the target organisms. Tergitol™ 7 is a surfactant that acts as a selective agent. The dual chromogens are incorporated to differentiate between *E. coli* and coliforms based on their enzymatic activity. IPTG induces β-D-galactosidase and agar is the gelling agent. *E.coli* possess both β-D-galactosidase and β-glucuronidase activity so appear dark blue to violet. Coliform bacteria that are not *E.coli* possess only β-D-galactosidase so appear pink to red. Any organism able to grow that does not belong to these groups appears colorless. This medium conforms to the performance requirements of ISO 9308-1 2014.

Typical Formulation

Enzymatic Digest of Casein	1.0 g/L
Yeast Extract	2.0 g/L
Sodium Chloride	5.0 g/L
Sodium Dihydrogen Phosphate	2.2 g/L
Di-Sodium Hydrogen Phosphate	2.7 g/L
Sodium Pyruvate	1.0 g/L
Sorbitol	1.0 g/L
Tryptophane	1.0 g/L
Tergitol™ 7	0.15 g/L
6-Chloro-3Indoxyl-β-D-Galactopyranoside	0.2 g/L
5-Bromo-4Chloro-3Indoxyl-β-D-Glucuronic Acid	0.1 g/L
Isopropyl-β-D-thiogalactophranoside (IPTG)	0.1 g/L
Agar	12.25 g/L

Final pH: 6.8 ± 0.2 at 25°C
Formula is adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. Refer to SDS

Preparation

1. Suspend 28.7 grams of the medium in one liter of purified water.
2. Bring rapidly to the boil with frequent agitation and temper in a water bath to 47°C.

Test Procedure

According to ISO 9308-1:2014, after filtration of the sample the membrane filter is placed on the surface of the agar ensuring that no air is trapped underneath. Invert the Petri dish prior to incubation. Incubate at 36±2°C for 21 ±3 hours.

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Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing and beige.

Prepared Appearance: Finished medium is clear to slight haze and yellow. Slight precipitate may be present.

Minimum QC:

Escherichia coli WDCM 00013	>70% Recovery, Dark-Blue to Violet Colonies
Escherichia coli WDCM 00012	>70% Recovery, Dark-Blue to Violet Colonies >70% Recovery, Pink to Red Colonies
Enterobacter aerogenes WDCM 00175	>70% Recovery, Pink to Red Colonies
Citrobacter freundii WDCM 00006	Total or Partial Inhibition
Enterococcus faecalis WDCM 00087	Total or Partial Inhibition
Enterococcus faecalis WDCM 00009	Colorless Colonies
Pseudomonas aeruginosa WDCM 00024	

Results

Growth Characteristics			
Organism	**β-D-galactosidase**	**β-D-glucuronidase**	**Color**
Escherichia coli	+	+	Dark Blue to Violet
Coliform bacteria	+	-	Pink to Red

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing or appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedures

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

ISO 9308-1:2014 Water quality – Enumeration of Escherichia coli and coliform bacteria. Part 1: Membrane filtration method for waters with low bacteria background flora.

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