

Chromocult® Coliform Agar

Selective agar for the simultaneous detection of total coliforms and *E. coli* in drinking water and processed food samples.

EPA approved.

Mode of Action

In the first instance, the interaction of selected peptones, pyruvate, sorbitol and phosphate buffer guarantees rapid colony growth, even for sublethally injured coliforms. The growth of Gram-positive bacteria as well as some Gram-negative bacteria is largely inhibited by the content of Tergitol® 7 which has no negative effect on the growth of the coliform bacteria.

For the second stage, Merck has developed a new combination of two chromogenic substrates which allow for the simultaneous detection of total coliforms and *E. coli*.

E. coli identification

The characteristic enzyme for coliforms, β -D-galactosidase cleaves the Salmon-GAL substrate and causes a salmon to red colour of the coliform colonies.

E. coli identification

The substrate X-glucuronide is used for the identification of β -D-glucuronidase, which is characteristic for *E. coli*.

E. coli cleaves both Salmon-GAL and X-glucuronide, so that positive colonies take on a dark-blue to violet colour. These are easily distinguished from other coliform colonies which have a salmon to red colour. As part of an additional confirmation of *E. coli*, the inclusion of tryptophane improves the indole reaction, thereby increasing detection reliability when it is used in combination with the Salmon-GAL and X-glucuronide reaction.

Typical Composition (g/litre)

Peptones 3.0; sodium chloride 5.0; sodium dihydrogen phosphate 2.2; di-sodium hydrogen phosphate 2.7; sodium pyruvate 1.0; tryptophane 1.0; agar-agar 10.0; Sorbitol 1.0; Tergitol® 7 0.15; 6-chloro-3-indoxyl-beta-D-galactopyranoside 0.2; isopropyl-beta-D-thiogalactopyranoside 0.1.

Preparation

Suspend 26.5 g in 1 litre of demin. water by heating in a boiling water bath or in free flowing steam. Stir the content to assist dissolution (approx. 35 mn). Some turbidity may occur, but this does not effect the performance!

■ **Do not autoclave! Do not overheat!**

pH: 6.8 ± 0.2 at 25 °C.

Note: After heat-treatment add *E. coli* / Coliform Supplement to the medium cooled to 45–50 °C if the sample material contains high gram-positive bacteria, *Pseudomonas* or *Aeromonas* spp.

The plates are opalescent and yellowish. Store at 4 ± 2 °C and protect from light. To prevent plates from becoming dry seal in plastic-pouches or bags.

Shelf-life under these conditions: 6 months.

Experimental Procedure and Evaluation

Inoculate the medium by the pour plate method or by spreading the sample material on the surface of the plates. In addition the membrane-filter-technique can also be used.

Incubation: 24 hours at 35 °C aerobically.

E. coli: dark-blue to violet colonies (Salmon-GAL and X-glucuronide reaction).

Total coliforms: salmon to red colonies (Salmon-GAL reaction) and dark-blue to violet colonies (*E. coli*).

Other Gram-negatives: colourless colonies, except for some organisms which possess β -D-glucuronidase activity. These colonies appear light-blue to turquoise.

In order to confirm *E. coli*, coat the dark-blue to violet colonies with a drop of KOVACS' indole reagent. If the reagent turns to a cherry-red colour after some seconds, a positive indole formation confirms the presence of *E. coli*.

Membrane-filter method:

The simultaneous detection of total coliforms and *E. coli* using Chromocult® Coliform Agar (CCA) relies on the production of specific colony colours. OSSMER et. al (1999) reported on the effect of the type and brand of membrane filters on the growth and colour formation of coliforms and *E. coli* on CCA. The best performance was obtained when using filters of cellulose-mixed-ester material, s. a. Gelman GN6 or Schleicher and Schüll ME25. For the validation of membrane filters it is advised to use one of these filters as reference.

Literature

FRAMPTON, E.W., RESTAINO, L. a. BLASZKO, L.: Evaluation of β -glucuronidase substrate 5-bromo-4-chloro-3-indol- β -D-glucuronide (X-GLUC) in a 24 hour direct plating method for *Escherichia coli*. – *J. Food Protection*, 51; 402-404 (1988).

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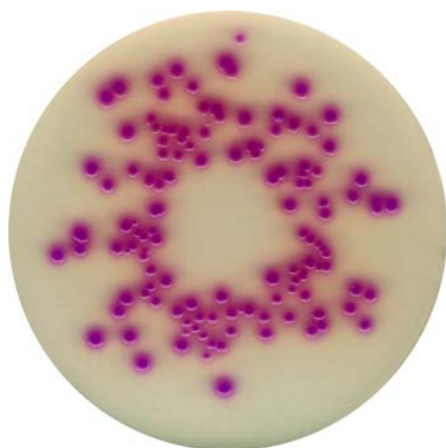
Chromocult® Coliform Agar

Ordering Information

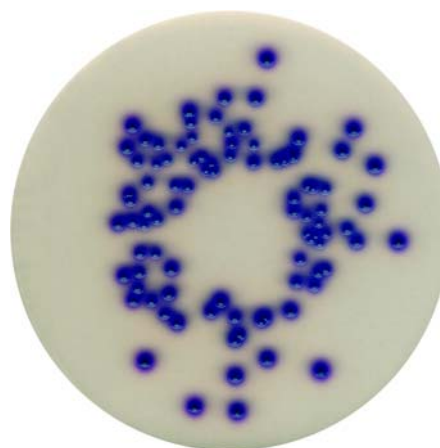
Product	Merck Cat. No.	Pack size
Chromocult® Coliform Agar	1.10426.0500	500 g
Bactident® Indole (dropper bottle)	1.11350.0001	1 x 30 ml
E. Coli/Coliform Selective-Supplement	1.00898.0001	1 x 16 vials
KOVÁCS Indole Reagent	1.09293.0100	100 ml
Cellulose-mixed-ester-GNG membrane filters	Gelman 66278	
Cellulose-mixed-ester-ME 25/21	Schleicher & Schüll 406870	

Quality control

Test strains	Recovery rate %	Growth	Colony colour	Salmon-GAL	X-Glucuronide	Indole
<i>Escherichia coli</i> ATCC 11775	≥ 70	good/very good	dark-blue to violet	+	+	+
<i>Citrobacter freundii</i> ATCC 8090	≥ 70	good/very good	salmon to red	+	-	-
<i>Escherichia coli</i> DSMZ 502	≥ 70	good/very good	blue to violet	+	-	+
<i>Salmonella enteritidis</i> ATCC 13076	not limited	fair/very good	colourless	-	-	-
<i>Enterococcus faecalis</i> ATCC 19433	≤ 0.01	none				



Citrobacter freundii
ATCC 8090



Escherichia coli
ATCC 11775