



# ChromoCult<sup>®</sup> Coliform Agar

For detection of coliforms in drinking  
water and processed food samples.



# ChromoCult® Coliform Agar



Cat.No. 1.10426.0500  
(500 g)

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

US-EPA approved, the approval by AOAC is pending

## Mode of action

Primarily the interaction of carefully selected peptones in addition to pyruvate, sorbitol and a phosphate buffer guarantees rapid colony growth, even for the sublethally injured coliforms.

The growth of accompanying Gram-positive bacteria flora as well as some Gram-negative bacteria flora is largely inhibited by the use of Tergitol®7 which has no negative effect on the growth of the coliform bacteria.

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

### Coliform identification

The characteristic enzyme for coliforms,  $\beta$ -D-galactosidase, cleaves the Salmon-GAL substrate and resulting in a salmon to red colouration of the coliform colonies.

### E.coli identification

The substrate X-glucuronide is used for the identification of  $\beta$ -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 the other coliform colonies which have a salmon to red colour. 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 as part of an additional confirmation for E.coli.

## Typical composition (g/litre)

Peptones 3.0; sodium chloride 5.0; sodium dihydrogen phosphate 2.2; disodium hydrogen phosphate 2.7; sodium pyruvate 1.0; tryptophan 1.0; Agar-agar 10.0; Sorbitol 1.0; Tergitol®7 0.15; chromogenic mixture 0.4.

## Preparation

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

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

**pH:  $6.8 \pm 0.2$  at 25 °C.**

**Note:** If the sample material contains high Gram-positive bacteria resp. Pseudomonas and Aeromonas spp. add E.coli/Coliform Supplement to the medium cooled to 45 - 50 °C. The plates are opalescent and yellowish. Store at  $4 \pm 2$  °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

Inoculate the medium by the pour-plate method or surface spreading. In addition the membrane-filter technique can also be used. Incubation: 24 hours at 35 - 37 °C.

## Evaluation

**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  $\beta$ -D-glucuronidase activity. These colonies appear light-blue to turquoise. In order to confirm E.coli, overlay the dark-blue to violet colonies with a drop of Kovacs® Indole reagent. If the reagent turns to a cherry-red colour after a few seconds the reaction is positive and 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 specific colouration of target colonies. Ossmer et. al (1999) reported 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 using filters of cellulose-mixed-ester material, e.g. Pall GN 6 or Schleicher and Schüll ME 25. It is advised to use one of these filters as reference for the validation of membrane filters.





Colour makes the difference.

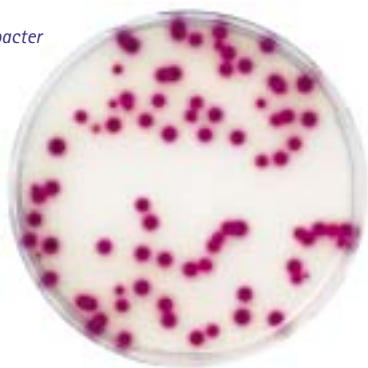
### Quality control

Test strains	Colony colour	Salmon-GAL	X-Glucuronide	Indol
Escherichia coli ATCC 11775	dark-blue to violet	+	+	+
Escherichia coli DSMZ 502	dark-blue to violet	+	+	+
Citrobacter freundii ATCC 8090	salmon to red	+	-	-
Salmonella enteritidis ATCC 13076	colourless	-	-	-
Enterococcus faecalis ATCC 19433	inhibited			

E.coli



Citrobacter



### Literature

- US-EPA approved method  
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Section 48: Product Test Methods – Enteric Indicator Organisms.

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### Additives

Cat. No.	Product	Pack size
1.09293.0100	KOVACS® Indole reagent	100 ml
1.11350.0001	Bactident® Indole	30 ml
1.00898.0001	E.coli/Coliform Selective Supplement	16 vials
1.10156.0001	Chromoplate® Coliform Agar	20 plates
Pall 66278	Cellulose-mixed-ester- GN6 membrane filters	
Schleicher & Schüll 406870	Cellulose-mixed- ester-ME 25/21	



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