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-gluconidase, 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 trypoptaphane 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 Schüß ME25.

For the validation of membrane filters it is advised to use one of these filters as reference.

Literature


## Chromocult® Coliform Agar

### Ordering Information

<table>
<thead>
<tr>
<th>Product</th>
<th>Merck Cat. No.</th>
<th>Pack size</th>
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<tbody>
<tr>
<td>Chromocult® Coliform Agar</td>
<td>1.10426.0500</td>
<td>500 g</td>
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<tr>
<td>Bactident® Indole (dropper bottle)</td>
<td>1.11350.0001</td>
<td>1 x 30 ml</td>
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<tr>
<td>E. Coli/Coliform Selective-Supplement</td>
<td>1.00898.0001</td>
<td>1 x 16 vials</td>
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<tr>
<td>KOVÁCS Indole Reagent</td>
<td>1.09293.0100</td>
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<tr>
<td>Cellulose-mixed-ester-GNG membrane filters</td>
<td>Gelman 66278</td>
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<td>Cellulose-mixed-ester-ME 25/21</td>
<td>Schleicher &amp; Schüll 406870</td>
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### Quality control

<table>
<thead>
<tr>
<th>Test strains</th>
<th>Recovery rate %</th>
<th>Growth</th>
<th>Colony colour</th>
<th>Salmon-GAL</th>
<th>X-Glucuronide</th>
<th>Indole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli ATCC 11775</td>
<td>≥ 70</td>
<td>good/very good</td>
<td>dark-blue to violet</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Citrobacter freundii ATCC 8090</td>
<td>≥ 70</td>
<td>good/very good</td>
<td>salmon to red</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>Escherichia coli DSMZ 502</td>
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<td>blue to violet</td>
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<td>Enterococcus faecalis ATCC 19433</td>
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</tbody>
</table>

![Citrobacter freundii ATCC 8090](image1.png)

![Escherichia coli ATCC 11775](image2.png)