

BRILLIANCE E. COLI / COLIFORM SELECTIVE AGAR

Code: CM1046

Brilliance™ E. coli/coliform Selective Agar (formerly Chromogenic E. coli/coliform Selective Agar) is for the detection and enumeration of Escherichia coli and other coliforms from food and water samples.

| Typical Formula* | gm/litre |
|---------------------------------|----------|
| Peptone | 8.0 |
| Di-sodium hydrogen phosphate | 2.2 |
| Sodium chloride | 5.0 |
| Potassium di-hydrogen phosphate | 1.8 |
| Sodium lauryl sulphate | 0.1 |
| Chromogenic mix | 0.35 |
| Agar | 10.6 |
| pH 6.7 ± 0.2 @ 25°C | |

* Adjusted as required to meet performance standards

Directions

Suspend 28.1g of *Brilliance E. coli/coliform Selective Agar* in 1 litre of distilled water. Bring the medium gently to the boil, to dissolve completely. Either pour the medium into sterile Petri dishes or keep molten at 45°C for pour plate technique.

Description

The recovery and enumeration of *Escherichia coli* and coliforms are important indicators of environmental and food hygiene. Detection of β-glucuronidase activity is widely used to differentiate *Escherichia coli* bacteria, as the enzyme, which is encoded by the uidA gene, is present in *Escherichia coli*, but not other members of the coliform group. As coliforms are lactose-positive, β-galactosidase activity, encoded by the lacZ gene, is then used to differentiate this group from other organisms able to grow on the selective medium. This results in purple *E. coli*, as they are able to cleave both chromogens, with other coliforms giving pink colonies as they cleave only the galactoside chromogen.

Brilliance E. coli/coliform Selective Agar contains two chromogenic agents:

- Rose-Gal: detects β-galactosidase activity
- X-Glu: detects β-glucuronidase activity.

The medium also contains sodium lauryl sulphate which acts as a selective agent, inhibiting the growth of Gram-positive organisms.

Most organisms in the coliform group are able to ferment lactose, so will cleave the pink Rose-Gal chromogen, producing pink colonies. *Escherichia coli* strains can be differentiated from the other coliforms as they also possess the enzyme β-glucuronidase (which has been shown to be highly specific to *Escherichia coli*). The X-Glu chromogen is targeted by this enzyme. The ability of *Escherichia coli* species to cleave both chromogens means that typical colonies will be purple (see Table 1).

Table 1: expected results, using *Brilliance E. coli/coliform Selective Agar*

| Organism | β - glucuronidase | β - galactosidase | Colony colour |
|-------------------------|-------------------------|-------------------------|--------------------|
| <i>Escherichia coli</i> | + | + | Purple |
| Coliforms | | + | Pink |
| Other organisms | - | - | Colourless or Blue |
| | + | - | |

Technique

Brilliance E. coli/coliform Selective Agar may be used for the detection and enumeration of *Escherichia coli* and coliforms in food and water samples.

Prepare food samples by diluting 1:5 or 1:10 (as appropriate) with 0.1% (w/v) sterile Peptone Water (CM0009), and homogenise in a Stomacher or a laboratory blender. Heavily contaminated water samples should first be diluted in Ringers Solution (BR0052) or Maximum Recovery Diluent (CM0733) so that the number of colonies to be counted is of a readable number e.g. 20-100 colonies. Potable water should be concentrated either by centrifugation or by using the filter membrane method.

The following techniques may be used:

1. Spread Plate

Dry the surface of the prepared plates. Pipette 0.1ml of the prepared sample onto the plate and spread over the surface with a sterile spreader. Incubate plates for 24 hours at 37°C.

2. Pour-Plate Method

Pipette 1ml of the prepared sample into an empty Petri dish. Add 15-20ml of medium, cooled to 45°C. Gently swirl the plates to thoroughly mix and allow to set. Incubate for 24 hours at 37°C.

3. Filter Membrane Method

Dry the surface of the prepared plates. Filter an appropriate volume of sample through the membrane. Place the membrane onto the surface of an agar plate and avoid trapping air-bubbles under the membrane. Incubate for 24 hours at 37°C.

For all methods count the numbers of pink and purple colonies. Multiply the numbers of colonies by the dilution factor and express the result as the number of coliforms and *Escherichia coli* per gram of food or volume of water.

Storage conditions and Shelf life

Dehydrated medium: store tightly capped in the original container at 10-30°C

Prepared medium will be stable for up to 2 weeks when stored at 2-8°C

Appearance

Dehydrated medium; light coloured, free-flowing powder

Prepared medium; light straw coloured, transparent gel

Quality control

| Positive controls: | Expected results |
|---|------------------------------|
| <i>Escherichia coli</i> ATCC®25922 * | Good growth; purple colonies |
| <i>Klebsiella pneumoniae</i> ATCC®13883 * | Good growth; pink colonies |
| Negative control: | |
| <i>Staphylococcus aureus</i> ATCC®25923 * | Inhibited |