67579 O157 Millichrome™ plus Agar

For the selective isolation and differentiation of E. coli O157 in food and animal samples.

Composition:

Ingredients	Grams/Litre
Peptone and yeast extract	13.0
Chromogenic mix*	1.2
Agar	15.0

Final pH 6.9 +/- 0.2 at 25°C

* confidential mix with chromogenic substrates

Store prepared media below 8°C, protected from direct light (max. 1 month). Store dehydrated powder, in a dry place, in tightly sealed containers at 2-25°C.

Preparation:

Step 1 (Preparation):

- Disperse slowly 29.2 g of powder base in 1 L of purified water.
- Stir until agar is well thickened.
- Heat and bring to boil (100 °C) while swirling or stirring regularly.

DO NOT HEAT TO MORE THAN 100 °C. DO NOT AUTOCLAVE AT 121 °C.

Warning: If using an autoclave, do so without pressure.

Advice 1: For the 100 °C heating step, mixture may also be brought to a boil in a microwave oven: after initial boiling, remove from oven, stir gently, then return to oven for short repeated bursts of heating until complete fusion of the agar grains has taken place (large bubbles replacing foam).

Advice 2: If a more selective, and more specific, medium is needed, add potassium tellurite solution (Cat. No. 17774) to obtain a final concentration of 2.5 mg/L at 45-50 °C.

Advice 3: In case of product samples containing a high load of Proteus, Cefixime can be added at 0.025 mg/L at 45-50 °C.

Advice 4: In case of product samples containing a high load of Pseudomonas and/or Aeromonas, Cefsulodin can be added at 5 mg/L at 45-50 °C.

Step 2 (Pouring plates):

- Cool in a water bath at 45-50 °C, swirling or stirring gently.
- Pour into sterile Petri dishes.
- Let it solidify and dry.

Inoculation:

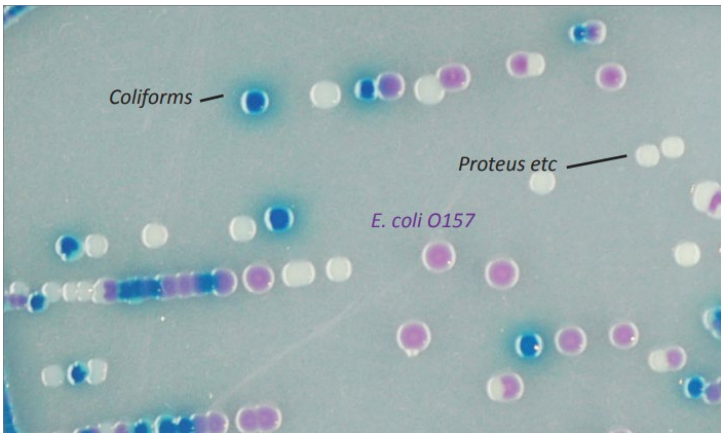
Samples are inoculated by direct streaking on the plate, or after enrichment step.

- If the agar plate has been refrigerated, allow to warm to room temperature before inoculation.
- Streak sample onto plate.

Principle and Interpretation:

Escherichia coli (*E. coli*) is a common intestine bacterium of humans and animals. Most strains of *E. coli* are harmless, but strains as verocytotoxigenic *E. coli* (VTEC), also known as shigatoxigenic *E. coli* (STEC) can cause severe foodborne diseases. Enterohaemorrhagic *E. coli* (EHEC) are a subset of VTEC,

which can cause severe disease in humans such as haemolytic uraemic syndrome. VTEC have been isolated from the gut of many animals, including cattle and sheep. VTEC are mainly transmitted to humans through contaminated foods. The *E. coli* serotype O157:H7 and its non-motile variant O157:H- are the most common VTEC serotype in relation to public health. [1]
O157 Millichrome™ plus Agar is easier for detection of *E. coli* O157 compared to SMAC. *E. coli* O157 shows a characteristic mauve colour after only 24 h of incubation, while most other E*. coli* are blue. The conventional medium for the detection of *E. coli* O157 is Sorbitol MacConkey (SMAC) Agar, which has very poor specificity, thus exhibiting an abundance of false positives (e.g. *Proteus* spp., *E. hermanii*, etc.). Sorbitol MacConkey Agar is also difficult to read because there is a change of colouration in the case of prolonged incubation.



Typical colony appearance

Peptone and yeast extract provide nitrogenous nutrients for growth and other essential growth factors. Sodium chloride is needed for the osmotic balance. Chromogenic mix contains chromogenic substrates for the color differentiation based on the ability to cleave the substrate by characteristic enzymes. If a more selective and specific, medium is needed, then it is recommended to add potassium tellurite supplement. If high load of *Proteus* spp. is expected, cefixime can be added. A high load of *Pseudomonas* and/or *Aeromonas* can be inhibited by addition of cefsulodin. Agar is added as the solidifying agent.

Limitation and further testing

- Sensitivity for *E. coli* O157 is 89% (Bettelheim et al. 1998). In absence of potassium tellurite, various non *E. coli* O157 may have same colony colour (like some *Salmonella* spp.).
- A latex confirmation test for O157 is suggested for suspect colonies. Definite identification as *E. coli* O157 requires, in addition to characterisation of O157 serotype, a final identification as *E. coli*.

Quality control:
Cultural characteristics after 24 h at 37°C under aerobically conditions.

Organisms (ATCC/WDCM)	Growth	Colony color
Escherichia coli O157:H7 (700728 /-)	+++	mauve
Escherichia coli O157:H7 (35150/-)	+++	mauve
Escherichia coli (25922/00013)	+++	metallic blue
Klebsiella pneumoniae (13883/00097)	+++	metallic blue
Enterococcus faecalis (29212/00087)	-	inhibited

References:
1. Shigatoxin/verocytotoxin-producing *Escherichia coli* (STEC/VTEC) infection - Annual Epidemiological Report 2016 [2014 data], Annual Epidemiological Report on Communicable Diseases in Europe (2016)
2. Method for enumerating *Escherichia coli* O157:H7 in compost and aged manure, U.S. Meat Animal Research Center USDA, ARS - Clay Center, Nebrask (2016)
3. B.D.Parsons et al., Detection, Characterization and Typing of Shiga Toxin-Producing Escherichia coli, frontiers in Microbiology (2016)
4. P. M. K. Njage, Detection, isolation and molecular characterisation of Shigatoxigenic O157 and non-O157 *Escherichia coli* in raw and fermented camel milk, African Journal of Microbiology Research Vol. 6(31), pp. 6031-6038 (2012)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.