67447 STEC Millichrome™ plus Agar Base

For detection of Shiga-Toxin producing *E.coli* (STEC)

Composition:

Ingredients	Grams/Litre
Peptone and yeast extract	8.0
Salts	5.2
Chromogenic mix*	2.6
Agar	15.0

Final pH 7.6 +/- 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. Rehydrated supplement is stable up to 2 month when stored at 2-8°C

Preparation:

Step 1 (Preparation of the base STEC Millichrome™ plus Agar base)

- Disperse slowly 30.8 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).

- Cool in a water bath to 45-50 °C. Swirl or stir gently to homogenize.

Step 2 (Preparation of the STEC Millichrome™ plus supplement and mix with base)

- Aseptically rehydrate 1 vial with 10 mL of sterile water.
- Swirl well until complete dissolution.
- Add 1 vial rehydrated solution to 1L STEC Millichrome™ plus Agar base cooled at 45-50°C.
- Swirl gently to homogenize.

Step 3 Pour plates

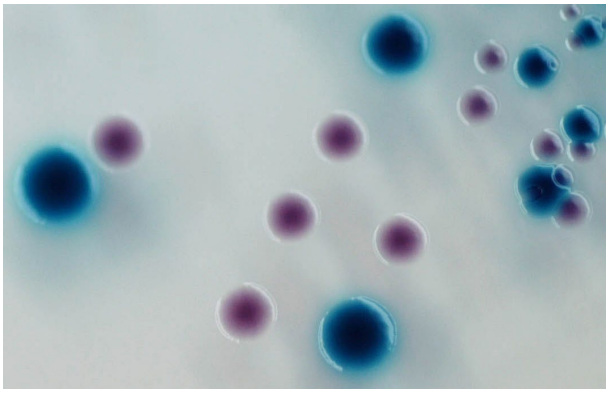
- Pour into sterile Petri dishes
- Let it solidify and dry.

Inoculation:

Samples are inoculated by direct streaking on the plate.

- If the agar plate has been refrigerated, allow to warm to room temperature before inoculation.
- Streak sample onto plate.
- Possible use of enrichment broth (for example TSB)

Principle and Interpretation:
Studies have shown that, non-O157 ShigaToxin-producing *E. coli* (STEC) have been responsible for foodborne poisoning outbreaks. The CDC has also reported warnings about this potential risk: "Disease caused by STEC ranges from self-limiting diarrhea to hemorrhagic colitis and hemolytic uremic syndrome (HUS). Serotype O157:H7, the most frequently implicated STEC causing hemorrhagic colitis and HUS, has been isolated from large foodborne outbreaks, as well as sporadic cases, in North America and abroad. However, 60 STEC serotypes have been implicated in diarrheal disease, and several non-O157:H7 serotypes have been implicated as the cause of foodborne outbreaks and HUS in the United States, Europe, and Australia. Studies from Canada, Europe, Argentina, and Australia suggest that non- O157:H7 STEC infections are as prevalent, or more so, than O157:H7 infection." [1]
In many cases, laboratories have limited their search for pathogenic *E. coli* to the common O157 serotype. This is due, among other reasons, to the fact that there were no available selective culture media for non-O157 E. coli.
STEC Millichrome™ plus Agar is a highly selective chromogenic culture medium intended to fill the above gap and for use in the qualitative direct detection, differentiation and presumptive identification of majority of Shiga-like-toxin producing *E. coli* (STEC), to aid in the diagnosis of STEC infections. Concomitant cultures are necessary to recover organisms for further microbiological testing or epidemiological typing. A lack of growth or the absence of mauve colonies on CHROMagar™ STEC does not preclude the presence of STEC. STEC Millichrome™ plus Agar is not intended to diagnose infection nor to guide nor monitor treatment for infections.
STEC Millichrome™ plus Agar can be used in the detection of STEC in the analyses of food products for human consumption, animal feed and in environmental samples. Excellent tool for large number of samples screening procedures.
STEC has mauve colonies, while other Enterobnacteriaceae show colorless or blue colonies or are inhibited. Gram positive bacteria are inhibited. STEC O157 is non fluorescent under UV light while other STEC serotypes are variable in fluorescence under the UV lamp at 365nm.



- STEC (mauve colonies)
- Other organisms (colorless, blue colonies or inhibited)

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. STEC Millichrome™ plus supplement contains selective agents which allows STEC to grow while other organisms are inhibited. Agar is the solidifying agent.

Limitation and further testing

- Some STEC could have a poor or no growth on the media.
- Some strains of non-STEC could appear as mauve colonies w/o fluorescence.
- Rare O157 are fluorescent positive.
- Final confirmation as STEC must be done by appropriate methods.
- Serotypes with agglutination tests can be performed directly from the colony.

Quality control:
Cultural characteristics after 18-24 h at 35-37°C under aerobic conditions.

Organisms (ATCC/WDCM)	Growth	Colony color
Escherichia coli O157 (35150/-)	+++	mauve
Escherichia coli O157(700728/00014)	+++	mauve
Enterococcus faecalis (29212/0008)	-	
Escherichia coli (25922/00013)	-	

References:

1. CDC report: "Prevalence of Non-O157:H7 Shiga Toxin-Producing Escherichia coli in Diarrheal Stool Samples [...]"
2. H. Glassman, C. Ferrato, L. Chui, Non-O157 Shiga Toxin producing Escherichia coli (STEC) in Alberta, Canada from 2018-2021, AMMI Canada – CACMIDM (2022)

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.