

INTENDED USE For laboratory use as a selective, chromogenic culture medium to aid in the detection, isolation and presumptive identification of verotoxin-producing strains of *Escherichia coli*, particularly serotype O157:H7.

SUMMARY OF TEST The Centers for Disease Control (USPHS-CDC) currently recommends that all clinical laboratories should culture for *E. coli* O157:H7 as a possible cause of diarrhea^{1,2}. Furthermore, because this bacterium is often endemic to cattle and spread through contaminated meat³, the Food Safety Inspection Service (USDA -FSIS) has declared it to be an “official adulterant” and requires meat producers to exclude it from their products.

Rainbow Agar O157 has both selective and chromogenic properties that make it particularly useful for isolating pathogenic *E. coli* strains. The medium contains chromogenic substrates that are specific for two *E. coli*-associated enzymes: β -galactosidase (a blue-black chromogenic substrate) and β -glucuronidase (a red chromogenic substrate).

- Strain O157:H7 is typically glucuronidase negative so it forms unique and distinctive black or gray colonies.
- Many other non-O157 toxigenic strains overproduce β -galactosidase relative to β -glucuronidase on this medium and consequently they are typically colored purple, violet or blue.
- Most non-pathogenic *E. coli* strains are glucuronidase positive, and range from pink to magenta with occasional purple or blue strains.
- Nearly all other bacterial species are either inhibited on this medium or they grow as white or cream-colored colonies.

CONTENTS This sealed bottle contains dry powder to make Rainbow Agar O157. 60 grams of powder will make 1 liter or approximately 50 plates.

PREPARATION This medium can be prepared in any volume to suit the user’s needs. To prepare 1 liter of Rainbow Agar O157, mix 60 grams of powder into 1 liter of purified water, boil gently to dissolve components and autoclave for 10 minutes at 15 lbs. pressure and 121°C. DO NOT exceed 10 minutes at this heat and pressure. For volumes less than 500 ml, autoclave for 5 minutes. Cool agar to 45-50°C before pouring or adding selective agents. If desired, add the appropriate amount of a filter sterilized stock solution of selective agent and mix well. Dispense approximately 20 ml of media into each petri plate. The medium is ready to use as soon as it has cooled, gelled and the surface has dried. The final medium should be clear and virtually colorless. No pH adjustment is needed. The final pH should be pH 7.9-8.3.

STORAGE Rainbow Agar O157 powdered medium is extremely hygroscopic and should be stored at 2°C to 25°C in a dry environment with the lid closed tightly. Unopened bottles may be stored in this manner until the expiration date on the label. Rainbow Agar O157 plates are stable for 2 weeks when stored in the dark, under refrigeration and packaged in cellophane to inhibit moisture loss.

USER QUALITY CONTROL The following organisms are recommended if quality control is desired or required. Inoculate Rainbow Agar O157 plates with the following strains by streaking for isolation, then incubate for 24 hours at 35°C without elevated CO₂. Colony color should be read from isolated colonies.

Organism	ATCC [®] number	Color on Rainbow Agar O157
<i>Escherichia coli</i> O157:H7	43894	Black or gray
<i>Escherichia coli</i>	11775	Pink or magenta
<i>Enterococcus faecalis</i>	19433	White

PROCEDURE—LOW BACKGROUND FLORA Inoculate the medium by streaking or spreading a sample suspected of containing

E. coli on the surface of the medium. Incubate the plates for 20 to 24 hours, or longer, at 35°C without elevated CO₂ and observe for the presence of colored colonies. The distinctive black or gray coloration of *E. coli* O157:H7 colonies is easily viewed by laying the petri plate against a white background. When O157 is surrounded by pink or magenta non-toxicogenic colonies, it may have a bluish hue. Upon sub-culturing, isolated *E. coli* O157:H7 colonies will have their typical black or gray coloration. Because of the selective nature of the medium, the size of the colonies formed is smaller than would be seen on other media commonly used to cultivate *E. coli*.

PROCEDURE—HIGH BACKGROUND FLORA To increase the selectivity of Rainbow Agar O157 use 0.8 mg/L potassium tellurite + 10 mg/L novobiocin. Tellurite is highly selective for *E. coli* O157:H7 and will reduce background flora considerably. Novobiocin inhibits *Proteus* swarming and the growth of tellurite-reducing bacteria. Caution must be used because rare strains of O157:H7 are tellurite sensitive⁵. *E. coli* O157:H7 colony coloration will be slightly bluer with these selective agents added.

EXPECTED RESULTS^a

Organism	Colony coloration ^b
<i>E. coli</i> O157:H7	Black or gray
<i>E. coli</i> O157:H7 glucuronidase positive	Purple-blue
<i>E. coli</i> O26:H11	Purple-magenta
<i>E. coli</i> O48:H21	Purple
<i>E. coli</i> O111:H- or O111:H8	Violet or gray
Non-toxicogenic <i>E. coli</i>	Pink or magenta ^c

^aColony coloration is indicative of isolated colonies.

^bColor can vary depending upon the *E. coli* strain tested.

^cCan be purple or blue.

LIMITATIONS This medium should not be used as the sole basis for identification of microorganisms. Any colony suspected of being *E. coli* O157 or another toxin producing serotype should be tested further to verify its identity using an approved confirmatory protocol.

Klebsiella pneumoniae and rare *Hafnia alvei* or *Citrobacter* strains may resemble *E. coli* O157. *Klebsiella* forms distinctive orange-gray colonies. *Citrobacter* forms blue-gray colonies and rare *Hafnia* strains may form light blue-gray to blue-black colonies. These species can be differentiated using the indole or PYR spot tests (*E. coli*: indole positive, PYR negative; *K. pneumoniae*, *H. alvei*: indole negative, *Citrobacter*: PYR positive).

REFERENCES

1. Centers for Disease Control and Prevention. 1993. Morbidity and Mortality Weekly Report 42:257-263.
2. Kay, B.A., P.M. Griffin, N.A. Strockbine and J.G. Wells. 1994. Clinical Microbiology Newsletter 16:17-19.
3. Doyle, M.P., and J.L. Schoeni. 1987. Applied Environmental Microbiology 53:2394-2396.
4. Okrend, A.J.G., B.E. Rose, and B. Bennett. 1990. Journal of Food Protection 53:249-255.
5. MacRae, M., T. Rebate, and I.D. Ogden. 1997. Letters in Applied Microbiology 25:135-137.

BIOLOG ORDERING INFORMATION

Rainbow Agar O157

Catalog Number	Description
80100	Dehydrated—60 g
80101	Dehydrated—500 g

Biolog Order Desk: 800-284-4949
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