

For detection of *Escherichia coli*

HiCrome™ EC Broth w/ RUG

Recommended for detection of *Escherichia coli* in water and food samples by a chromogenic and fluorogenic method

M2073

Composition **

Ingredients	Grams/Litre
Yeast extract	2.00
Acicase#	1.00
Buffers	4.50
Sodium chloride	0.50
Salts	2.45
Chromogenic mixture	0.112

Final pH (at 25°C) 7.0 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Equivalent to Casein acid hydrolysate

Directions

Suspend 10.56 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Dispense into sterile tubes or flasks as desired.

Principle and Interpretation

Escherichia coli is a member of faecal coliform group of bacteria. It is a member of the indigenous faecal flora of warmblooded animals. *E. coli* is considered a specific indicator of faecal contamination and the possible presence of enteric pathogens. *E. coli* can be reliably detected with media that contain a chromogenic or fluorogenic substrate for beta glucuronidase, an enzyme that occurs almost exclusively in *E. coli*.

▲ Resorufin-beta-D-glucuronic acid methyl ester (RUG) is a highly sensitive chromogenic and fluorogenic indicator for *E. coli*. In contrast to MUG, RUG is more specific and does not require fluorescent detection. The released dye Resorufin itself gives intense pink color which can be visually detected. Additional confirmation can be done by observation of fluorescence under uv light.

Yeast extract and Acicase provides carbonaceous, nitrogenous substances, long chain amino acids, vitamins and other essential nutrients. Sodium chloride maintains osmotic equilibrium. The medium has a strong buffering system to control the pH in the medium. Gram-positive bacteria especially *Staphylococcus*, *Bacillus* species and faecal *Streptococcus* are inhibited.

Type of specimen

Food sample; Water samples

Specimen Collection and Handling

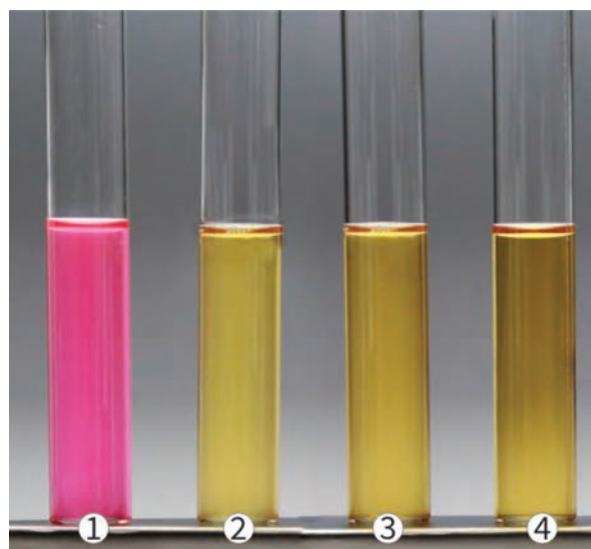
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (1).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (2).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in



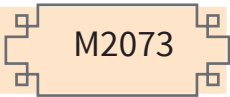
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1. *E. coli* ATCC 25922
2. *P. aeruginosa* ATCC 27853
3. *S. Typhimurium* ATCC 14028
4. Control

▲ Resorufin-beta-D-glucuronic acid methyl ester (RUG) is a patent of BIOSYNTH

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individual safety data sheets.

Limitations

1. Slight variation in intensity of colour may be observed depending on the isolates

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

- Appearance of powder** : Pale yellow to orange homogeneous free flowing powder.
- Colour and Clarity of prepared medium** : Yellow coloured clear solution without any precipitate.
- Reaction** : Reaction of 1.05% w/v aqueous solution at 25°C. pH : 7.0±0.2
- Cultural Response** : Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Colour of medium	*Fluorescence (at 366 nm)
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	bright pink	positive, throughout the tube
<i>Escherichia coli</i> ATCC 10536	50-100	luxuriant	bright pink	positive, throughout the tube
<i>Citrobacter freundii</i> ATCC 8090	50-100	luxuriant	pale yellow	negative
<i>Salmonella</i> Enteritidis ATCC 10376 (00030*)	50-100	luxuriant	pale yellow	negative
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	≥10 ³	inhibited	no colour change	
<i>Bacillus subtilis</i> subsp. <i>spizizenii</i> ATCC 6633 (00003*)	≥10 ³	inhibited	no colour change	

Key : * : Corresponds to WDCM number

Storage and Shelf-life

Store dehydrated powder and prepared medium on receipt at 2-8°C. Use before expiry period on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3, 4).

References

1. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.