69429 EC RUG™ ChromoSelect Broth

For the detection of *E. coli* by the chromogenic and fluorogenic substrate resorufin-beta-D-glucuronic acid methyl ester which has several advantages compared to MUG. *E. coli* appears as bright pink colony and is red fluorescence at 366 nm light source. In contrast to MUG, RUG is more specific and does not require fluorescent detection.

Composition:

Ingredients	Grams/Litre
Yeast extract	2.0
Casein acid hydrolysate	1.0
Buffers	4.5
Sodium chloride	0.5
Salts	2.45
Chromogenic mixture	0.112
Final pH 7.0 +/- 0.2 at 25 °C	

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

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 β-glucuronidase, an enzyme that occurs almost exclusively in *E. coli*.
Resorufin-β-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 Casein acid hydrolysate provide nitrogenous compounds, vitamins, carbon, sulphur and amino acids. Sodium chloride maintains osmotic balance. The medium has a strong buffering system to control the pH in the medium. The salt contains sodium lauryl sulphate, which inhibits gram-positive bacteria especially Staphylococci, *Bacillus* species and faecal Streptococci.

Cultural characteristics after 18-24 hours at 35-37 ºC.

Organisms (ATCC/WDCM)	Inoculum (cfu)	Growth	Color of Medium	Fluorescence at 366 nm
Escherichia coli (25922/00013)	50-100	+++	bright pink	+
Escherichia coli (10536/-)	50-100	+++	bright pink	+
Citrobacter freundii (8090/-)	50-100	+++	pale orange	-
Salmonella Enteritidis (10376/00030)	50-100	+++	pale orange	-
Staphylococcus aureus subsp. aureus (25923/00034)	$\geq 10^3$	-	no colour change	
Bacillus subtilis subsp. spizizenni (6633/00003)	$\geq 10^3$	-	no colour change	

References:

1. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, 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)