Violet Red Bile Agar with MUG

Intended Use
Violet Red Bile Agar with MUG is used as a selective medium for the detection and enumeration of coliform organisms by a fluorogenic procedure.

Summary
Escherichia coli is used as an indicator organism to determine unsanitary conditions. A number of selective media are recommended for use in enrichment, presumptive identification and confirmatory procedures for demonstrating the presence of coliforms. These procedures require longer incubation period. Violet Red Bile Agar is recommended by APHA for the detection and enumeration of coliforms in foods and dairy products. Addition of MUG to this medium permits the rapid detection of *E. coli*, when the medium is observed for fluorescence under UV light, requiring no further confirmation. *E. coli* possesses the enzyme beta-glucuronidase which specifically cleaves MUG to form a fluorogenic compound 4-methylumbelliferone, which results in visible blue-green fluorescence. MUG Violet Red Bile Agar is therefore recommended for the specific detection of *E. coli*.

Principle
Peptone, yeast extract and lactose provide essential nutrients. Crystal violet and bile salts inhibit some Gram-positive and Gram-negative bacteria. Neutral red acts as a pH indicator and helps to exhibit red colonies in the presence of acid from lactose fermentation. Acidic pH decreases the intensity of fluorescence, thus making it difficult to identify fluorescent *E. coli*. The plates after primary identification i.e. red colonies surrounded by bile precipitate were exposed to ammonia fumes to increase fluorescence as suggested by Freir and Hartman. The substrate MUG is hydrolyzed by an enzyme beta-glucuronidase, which is present in most of *E. coli* and a few strains of *Salmonella*, *Shigella* and *Yersinia* to yield a fluorescent end product, 4-methylumbelliferone. *Proteus vulgaris* in large numbers may suppress gas production by *E. coli*.

Formula*

Ingredients	**g/L**
Yeast Extract	3.0
Peptone	7.0
Lactose	10.0
Bile Salt No.3	1.5
Sodium Chloride	5.0
Neutral Red	0.03
Crystal Violet	0.002
MUG- (4-methylumbelliferyl -B-D-glucuronide)	0.1
Agar	15.0
Final pH (at 25°C)	7.4 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Specimen Collection and Handling
Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions
1. Suspend 41.63 g of the powder in 1000 mL purified / distilled water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely. DO NOT AUTOCLAVE.
4. Cool to 45°C-50°C and use immediately.

Quality Control

Dehydrated Appearance: Pinkish beige coloured, homogenous, free flowing powder.
Prepared Appearance: Reddish purple, clear to slightly opalescent gel, with or without slight precipitate forms in petridishes.
Growth Promotion Test: Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP/IP and growth is observed after an incubation at 30°C-35°C for 18 to 24 hours.
Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time specified in the test, inoculating ≤ 100 cfu of appropriate microorganism at 30°C-35°C for 18 hours.
Indicative Properties: The test results observed are within the specified temperature and time, inoculating ≤100 cfu of appropriate microorganism.
Inhibitory Properties: No growth of the test microorganism occurs for the specified temperature and not less than the longest period of the time specified, inoculating > 100 cfu of the appropriate microorganism at 30°C-35°C for 24 hours.

Organism (ATCC)	**Growth**	**Colour of Colony**	**Fluorescence**
Escherichia coli (25922)	Good	Deep red with bile precipitate	+
Klebsiella aerogenes (13048)	Good	Pink	-
Inhibitory			
Staphylococcus aureus subsp. *aureus* (25923)	Inhibited	-	-

Note:
For good growth – Growth obtained on test media should not differ by a factor greater than 2 from calculated value for a standardized inoculum.
For inhibition no growth of test microorganism should occur.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.
2. Marshall, (Ed.), 1985, Standard Methods for the Examination of Dairy Products, 16th Ed., APHA, Washington, D.C.
3. Feng P. C. S. and Hartman P. A., 1982, Appl. Environ. Microbiol., 43 :1320.
4. FDA Bacteriological Analytical Manual, 8th Edi, AOAC International, Gaithersburg.
5. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	**Product Description**	**Pack Size**
201220030100	Dehydrated Culture Media	100 g
201220030500	Dehydrated Culture Media	500 g

Disclaimer
Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.