remel
MUG DISK

REF R21135 ..... 25 Disks/Vial

1. INTENDED USE

Remel MUG Disk is a reagent-impregnated disk recommended for use in qualitative procedures for rapid, presumptive identification of Escherichia coli.

2. SUMMARY AND EXPLANATION

In 1976, Kilian and Bulow reported the enzyme β-gluc-uronidase is present in most strains of E. coli (97%).[1] Godsey et al. supported their findings in an evaluation of methods based on microbial enzyme activity profiles.[2] They found the substrate, 4-methylumbelliferyl-β-D-glucuronide (MUG), to be both sensitive and selective for detection of β-gluc-uronidase activity. Trepeta and Edberg used the MUG test in conjunction with oxidase, indole, and lactose fermentation for rapid, cost effective identification of E. coli.[3]

3. PRINCIPLE

β-D-glucuronidase is an enzyme produced by most strains of E. coli. This enzyme cleaves the substrate, 4-methylumbelliferyl-β-D-glucuronide (MUG) and thereby produces a fluorescent end product called methylumbelliferone.4 This compound is detectable using a long wave ultraviolet light source. In the indole test, the enzyme tryptophanase attacks the tryptophan molecule on its side chain, leaving the aromatic ring in the form of indole. The indole is then detected by addition of ρ-dimethylamino-benzaldehyde (Kovacs' Reagent) which produces a red color.

4. REAGENTS

Reactive Ingredients:

4-methylumbelliferyl-β-D-glucuronide

Tryptophan

5. PRECAUTIONS

This product is for In Vitro diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

6. STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature (20-25°C) before use. Protect product from light, as it is light sensitive.

7. PRODUCT DETERIORATION

This product should not be used if (1) the disk color has changed from white, (2) the expiration date has passed, (3) the desiccant has changed from blue to pink, or (4) there are other signs of deterioration. Protect disks from moisture by removing from the vial only those disks necessary for testing. Promptly replace the cap and return the vial to 2-8°C.

8. SPECIMEN COLLECTION, STORAGE, TRANSPORT

Specimens should be collected and handled following recommended guidelines.[5,6]

9. MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Forceps, (7) Longwave ultraviolet light, (8) Kovacs' Indole Reagent (REF R21227), (9) Petri dish, filter paper, test tube, (10) Demineralized water.

10. PROCEDURE

Method A (Direct Disk Test):

1. Place a MUG Disk in an empty sterile petri dish and add one drop of demineralized water. Alternatively, the disk can be placed directly on the agar surface, in which case, it will not require the addition of water because moisture from the medium will rehydrate the disk.
2. Smear 2-3 isolated colonies on the disk.
3. Place a piece of filter paper saturated with water in the lid of the petri dish to provide a humid environment.
4. Incubate aerobically for up to 30 minutes at 35-37°C.
5. Following incubation, examine the disk for fluorescence using a longwave ultraviolet light (360 nm) in a darkened room. Note: The MUG reaction must be interpreted before adding Kovacs' Indole Reagent.
6. After reading the disk for fluorescence, add one drop of Kovacs' Indole Reagent to the disk. Immediately observe for a red color development.

Method B (Tube Test):

1. Add 0.25 ml of demineralized water to a clean, plastic or glass tube.
2. Make a heavy suspension of the test isolate (3-5 colonies from an 18-24 hour blood agar plate) in the tube. Note: A second tube may be inoculated with a known MUG-negative organism to serve as a negative control and aid in the interpretation of the test.
3. Using forceps place a MUG Disk in the tube and shake vigorously to ensure adequate elution of the substrate in the surrounding liquid.
4. Incubate aerobically for 1 hour at 35-37°C.
5. Following incubation, examine the tube for fluorescence using a longwave ultraviolet light (360 nm) in a darkened room. Note: The MUG reaction must be interpreted before adding Kovacs' Indole Reagent.
6. After reading the tube for fluorescence, add 2-3 drops of Kovacs' Indole Reagent to the tube and mix. Immediately observe for a red color development.

11. INTERPRETATION

MUG Disk Test (Direct or Tube Test):

Positive Test	Blue fluorescence
Negative Test	No fluorescence

Indole Test:

Positive Test	Red color development on the disk or in the tube
Negative Test	No red color development on the disk or in the tube

12. QUALITY CONTROL

All lot numbers of MUG Disk have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Escherichia coli ATCC® 25922	Aerobic, 30-60 min @ 35-37°C	MUG (+) Indole (+)
Klebsiella pneumoniae ATCC® 27736	Aerobic, 30-60 min @ 35-37°C	MUG (-) Indole (-)
Proteus mirabilis ATCC® 12453	Aerobic, 30-60 min @ 35-37°C	MUG (-) Indole (-)

13. LIMITATIONS

1. This test is only part of the overall scheme for the identification of E. coli. Further biochemical and/or serological testing is required for definitive identification. Consult appropriate references for further instructions.[5,6]
2. Most strains of E. coli O157:H7 are MUG-negative. The use of an E. coli 0157:H7 latex agglutination test in conjunction with the MUG test is recommended for rapid identification of isolates during outbreaks.[4,5]
3. Some strains of Shigella are MUG-positive. Serological testing may be required to differentiate Shigella and E. coli.
4. False-negative reactions have been reported with inoculum removed from MacConkey agar and triple sugar iron agar.[4]
5. Organisms other than E. coli (e.g., Salmonella, Shigella, Staphylococcus, Streptococcus, etc.) possess the enzyme β-glucuronidase and are MUG-positive. Testing only lactose-positive, gram-negative rods for β-glucuronidase activity helps preclude misidentification of other organisms as E. coli.[7]

14. BIBLIOGRAPHY

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15. PACKAGING

REF R21135, MUG Disk..... 25 Disks/Vial

16. SYMBOL LEGEND

REF	Catalogue Number
IVD	*In Vitro* Diagnostic Medical Device
	Consult Instructions for Use (IFU)
	Temperature Limitations (Storage temp.)
LAB	For Laboratory Use Only
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)
	Manufactured by

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