



MOTILITY INDOLE LYSINE MEDIUM (MIL)

- For in vitro use only -

Catalogue No. TM63

Our MIL Medium is a semi-solid medium used in the presumptive identification and differentiation of *Enterobacteriaceae*.

MIL Medium allows for characterization and differentiation between members of the *Enterobacteriaceae* family based on four criteria: motility, lysine decarboxylation, lysine deamination, and indole production. Our formulation is based on the work of Reller and Mirrett whom first reported the usefulness of this new medium through extensive testing using enteric cultures in 1975.

The semi-solid nature of MIL Medium allows for motility detection since motile organisms can grow and radiate from the central inoculation stab line. Non-motile organisms cannot diffuse into the medium and will only exhibit growth along the stab. Indole production determination is possible since pancreatic digest of casein is rich in the amino acid tryptophan. Tryptophan is an amino acid that can be oxidized by some bacteria to form three major end products: indole, pyruvic acid, and ammonia. Detection of indole indicates tryptophan degradation and can be accomplished by the addition of certain aldehydes, such as p-dimethylaminobenzaldehyde contained in Kovacs Reagent, to form a color end product. In this case, Kovacs Reagent reacts with indole to form a pinkish-red end product that is highly visible.

The addition of the amino acid, lysine, along with the pH indicator, bromcresol purple, allows for the detection of the bacterial enzymes lysine decarboxylase and lysine deaminase. Decarboxylation is the process in which bacteria that possess specific enzymes can attack amino acids at their carboxyl ends. Organisms possessing the enzyme, lysine decarboxylase, can breakdown lysine to yield the alkaline end product cadaverine. This alkaline shift in the pH is detected by bromcresol purple that changes to purple when sufficient cadaverine is produced. The lysine deamination reaction can also be observed in this

medium as a red color change at the top of the medium. This red coloration occurs by an unknown mechanism whereby one of the final end products from deamination reacts with bromcresol purple.

Formula per Litre of Medium

Peptone	10.0 g
Pancreatic Digest of Casein	10.0 g
Yeast Extract	3.0 g
Lysine	10.0 g
Dextrose	1.0 g
Ferric Ammonium Citrate	0.5 g
Bromcresol Purple	0.02 g
Agar.....	2.0 g

pH 6.6 ± 0.2

Recommended Procedure

1. Allow medium to adjust to room temperature prior to inoculation.
2. Take a well-isolated colony from a pure culture plate and pick the centre using a straight inoculating needle.
3. Inoculate by stabbing the middle of the tube $\frac{3}{4}$ the depth of the medium.
4. Incubate tubes aerobically at 35°C with loose caps.
5. Examine tubes and interpret results after 18 to 24 hours of incubation. Interpret motility and lysine decarboxylation and deamination reactions prior to adding the Kovacs reagent.
6. For the indole test, add 4 drops of Kovacs Reagent (Cat No. RK75) and read results within 1 minute. Do not shake; the reagent should remain at the surface of the medium.

Interpretation of Results

Motility:

Positive (+): Diffuse growth radiating outside of the stab line

Negative (-): Growth only along the stab line

Lysine Decarboxylation:

Positive (+): Purple color throughout the medium. The purple coloration may vary in intensity due to reduction of the indicator

Negative (-): Yellow color throughout the medium with or without a purple or red top

Lysine Deamination:

Positive (+): Red or red-brown color at the top centimetre of the medium

Negative (-): No red color at the top

Indole Production:

Positive (+): Red color change after the addition of Kovacs reagent

Negative (-): No color change after the addition of Kovacs reagent; remains yellow

Quality Control

After checking the medium for correct pH, colour, depth, and sterility, the following organisms are used to determine the performance of the completed medium.

Organism	Expected Results			
	MOT	IND	LDC	LDA
<i>Escherichia coli</i> ATCC 25922	+	+	+	-
<i>Proteus mirabilis</i> ATCC 12453	+	-	-	+
<i>Shigella flexneri</i> ATCC 12022	-	-	-	-

Storage and Shelf Life

Our MIL Medium should be protected from light and stored in an upright position at 4°C to 8°C. Under these conditions the medium has a shelf life of 16 weeks from the date of manufacture.

Ordering Information

Cat#	Description	Format
TM63	MIL Medium [13x100-mm with screw cap]	10/pkg
RK75-25	Kovacs Reagent 25-mL	Each

References

1. Reller LB, Mirrett S. Motility-indole-lysine medium for presumptive identification of enteric pathogens of *Enterobacteriaceae*. J Clin Microbiol 1975; 2:247-52.
2. Ederer GM, Lund ME, Balazevic DJ, Reller LB, Mirrett S. Motility-indole-lysine-sulfide medium. J Clin Micro 1975; 2:266-7.
3. MacFaddin JF. Media for isolation-cultivation-maintenance of medical bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
4. MacFaddin JF. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Lippincott Williams and Wilkins. Philadelphia, 2000.

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