KBM001 HiMotility™ Biochemical kit for *Escherichia coli*

**Introduction**

KBM001 is a comprehensive test system that can be used for identification and differentiation of *Escherichia coli*. *Escherichia coli* are gram-negative, lactose fermenting coccobacillary rods which are frequently isolated from food, feces, water and other relevant clinical samples. HiE.Coli identification kit can be used for screening pathogenic organisms and can also be used for validating known laboratory strains. The complete list of organisms that can be identified with this system is given in the identification index provided with the kit.

**Principle**

Each KBM001 kit is a standardized colorimetric identification system utilizing seven conventional biochemical tests including motility and four carbohydrate utilization tests. The tests are based on the principle of pH change and substrate utilization. On incubation *E.coli* exhibit metabolic changes which are indicated by a spontaneous color change in the media that can be either interpreted visually or after addition of reagent wherever required.

**Kit contents**

1. Each kit contains sufficient material to perform 10 tests.
2. 10 kits of KBM001.
3. Technical product insert.
4. Result Interpretation Chart and Result Entry Datasheet.

**Instructions for use**

1. **Preparation of inoculum**
   - KBM001 cannot be used directly on clinical specimens. The organisms to be identified have to be first isolated and purified. Only pure cultures should be used.
   - Isolate the organism to be identified on a common medium like Nutrient Agar (M001) or a differential medium like MacConkey Agar (M082). Pick up a single well isolated colony and inoculate in 5ml Brain Heart Infusion broth and incubate at 35-37°C for 4-6 hours until the inoculum turbidity is ≥ 0.1OD at 620nm or 0.5 McFarland standard. Alternatively, a homogeneous suspension made in 2-3 ml sterile saline can be used for inoculation. The density of the suspension should be adjusted to 0.1OD at 620nm or 0.5 McFarland standard.
   - Note: Erroneous false negative results may be obtained if the inoculum turbidity is less than 0.1 OD.

2. **Inoculation of the kit**
   - Open the kit aseptically. Peel off the sealing foil.
   - Stab inoculate the 1st well. DO NOT INOCULATE THE 2nd WELL.
   - Inoculate the remaining kit (well no. 3-12) by stabbing each individual well (except well no. 2) with a loopful of inoculum. Inoculum should reach the bottom of the wells.


**Interpretation of results**

Interpret results as per the standards given in the Result Interpretation Chart.

**Motility : Well No. 1**
- Motility is seen as movement of bluish green growth from 1st well to 2nd well.

**Indole Test : Well No. 3**
- Add 1-2 drops of Kovac’s reagent (R008).
- Development of reddish pink colour within 10 seconds indicates positive reaction.
- Reagent remains pale coloured if the test is negative.

**Nitrate Reduction Test : Well No. 6**
- Add 1-2 drops of Sulphanilic acid (R015) and 1-2 drops of N,N-Dimethyl-1-Napthylamine Reagent (R009).
- Immediate development of pinkish red colour on addition of reagent indicates positive reaction.

Please refer disclaimer Overleaf.
Identification Index of various *Escherichia* species

<table>
<thead>
<tr>
<th>No.</th>
<th>Test</th>
<th>Reagents to be added after incubation</th>
<th>Principle</th>
<th>Original colour of the medium</th>
<th>Positive reaction</th>
<th>Negative reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Motility</td>
<td>-</td>
<td>-</td>
<td>Colourless</td>
<td>Bluish green</td>
<td>No growth seen</td>
</tr>
<tr>
<td>2</td>
<td>Motility</td>
<td>-</td>
<td>Detects motility</td>
<td>Colourless</td>
<td>movement of bluish green growth seen</td>
<td>No growth seen</td>
</tr>
<tr>
<td>3</td>
<td>Indole</td>
<td>1-2 drops of Kovac’s indole reagent</td>
<td>Detects deamination of tryptophan</td>
<td>Colourless</td>
<td>Reddish pink</td>
<td>Colourless</td>
</tr>
<tr>
<td>4</td>
<td>Citrate utilization</td>
<td>-</td>
<td>Detects capability of organism to utilize citrate as a sole carbon source</td>
<td>Green</td>
<td>Blue</td>
<td>Green</td>
</tr>
<tr>
<td>5</td>
<td>Glucuronidase</td>
<td>-</td>
<td>Detects Glucuronidase activity</td>
<td>Colourless</td>
<td>Yellow</td>
<td>Colourless</td>
</tr>
<tr>
<td>6</td>
<td>Nitrate reduction</td>
<td>1-2 drops of sulfanilic acid and 1-2 drops of N,N-Dimethyl-1-Napthylamine</td>
<td>Detects Nitrate reduction</td>
<td>Colourless</td>
<td>Pinkish red</td>
<td>Colourless</td>
</tr>
<tr>
<td>7</td>
<td>ONPG</td>
<td>-</td>
<td>Detects β-galactosidase activity</td>
<td>Colourless</td>
<td>Yellow</td>
<td>Colourless</td>
</tr>
<tr>
<td>8</td>
<td>Lysine utilization</td>
<td>-</td>
<td>Detects Lysine decarboxylation</td>
<td>Drive green to Light Purple</td>
<td>Purple / Dark Purple</td>
<td>Yellow</td>
</tr>
<tr>
<td>9</td>
<td>Lactose</td>
<td>-</td>
<td>Lactose utilization</td>
<td>Pinkish Red / Red</td>
<td>Yellow</td>
<td>Red / Pink</td>
</tr>
<tr>
<td>10</td>
<td>Glucose</td>
<td>-</td>
<td>Glucose utilization</td>
<td>Pinkish Red / Red</td>
<td>Yellow</td>
<td>Red / Pink</td>
</tr>
<tr>
<td>11</td>
<td>Sucrose</td>
<td>-</td>
<td>Sucrose utilization</td>
<td>Pinkish Red / Red</td>
<td>Yellow</td>
<td>Red / Pink</td>
</tr>
<tr>
<td>12</td>
<td>Sorbitol</td>
<td>-</td>
<td>Sorbitol utilization</td>
<td>Pinkish Red / Red</td>
<td>Yellow</td>
<td>Red / Pink</td>
</tr>
</tbody>
</table>

Note: Based on % strains showing reactions following symbols have been assigned from laboratory results and standard references.

+  = Positive (more than 90%)
-  = Negative (more than 90%)
V  = Variable (11-89%)

**Result Interpretation chart**

**Important points to be taken into consideration while interpreting the result**

1. In case of Carbohydrate fermentation test some microorganisms may show weak reaction. In this case record the reaction as ± and incubate further up to 48 hours. Orange colour after 48 hours of incubation should be interpreted as a negative reaction.
2. In case of Lysine utilization, incubation up to 48 hours may be required.
3. At times organisms give contradictory result because of mutation or the media used for isolation, cultivation and maintenance.
4. The identification index has been compiled from standard references and results of tests obtained in the laboratory.

**Precautions**

- Clinical samples and microbial cultures should be considered potentially pathogenic and handled accordingly.
- Aseptic conditions should be maintained during inoculation and handling of the kits.

**Disposal of used material**

After use, kits and the instruments used for isolation and inoculation (pipettes, loops etc.) must be disinfected using a suitable disinfectant and then discarded by incineration or autoclaving in a disposable bag.

**Storage and Shelf-life**

On receipt store between 2-8 °C. Shelf-life is 12 months.

**Disclaimer:**

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.