

## KB013 HiBacillus™ Identification Kit

### Introduction

KB013 is a combination of 12 tests for identification of *Bacillus* species. (Kit contains sterile media for Malonate, Voges Proskauer's Citrate, ONPG, Nitrate reduction, Catalase, Arginine and 5 different carbohydrates utilization tests - Sucrose, Mannitol, Glucose, Arabinose, Trehalose).

Reagents supplied with kit : Nitrate Reagents : - Naphthylamine Solution (R009) and Sulphanilic Acid 0.8% (R015), Barritt Reagent A (R029) and Barritt Reagent B (R030) for VP Test

*Bacillus* is a genus of gram positive bacilli. It is major cause of food poisoning and therefore of clinical significance. KB013 can be used for screening food samples and other relevant clinical samples. It can also be used for validating known laboratory strains. The complete list of organisms that can be identified with this kit is given in the identification index provided with the kit.

### Principle

Each KB013 is a standardized, colorimetric test system based on carbohydrate utilization and other biochemical tests specific for the identification of *Bacillus* species. The tests are based on the principle of pH change and substrate utilization. *Bacillus* species on incubation exhibit metabolic changes which are indicated by a colour change in the media that can be either interpreted visually or after addition of reagent wherever required.

### Kit Contents

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| 1. Each kit contains sufficient material to perform 10 tests. | 6. Sulphanilic acid 0.8%(R015)                |
| 2. 10 kits of KB013   | 7. N,N-Dimethyl-1-Naphthylamine reagent(R009) |
| 3. Technical product insert                                   | 8. Baritt reagent A(R029)                     |
| 4. Result Interpretation Chart and Result Entry Datasheet     | 9. Baritt reagent B(R030)                     |
| 5. Identification Index                                       |   |

### Instructions for use

#### Preparation of inoculum

- KB013 cannot be used directly on clinical or food samples. The organism to be identified has to be first isolated and purified. Pick up a single isolated colony and inoculate in 5 ml Brain Heart Infusion Broth (M210) and incubate at 35-37°C for 6 to 8 hours until inoculum turbidity is  $\geq 1.0$  OD at 620nm.

**Note :** Erroneous false negative result may be obtained if the inoculum turbidity is less than 1.0 OD.

#### Method of Inoculation :

- Open the kit aseptically. Peel off the sealing foil.
- Inoculate each well with 50 $\mu$ l of the above inoculum by surface inoculation method
- Alternately, the kit can also be inoculated by stabbing each individual well with a loopful of inoculum.

**Incubation :** Temp. of Incubation : 35-37°C. Duration of Incubation : 24-48 hrs.

### Interpretation of results

- Interpret results as per the standards given in the Result Interpretation Chart. Addition of reagents in well no 2, 5 should be done at the end of incubation period that is after 24 to 48 hours.

#### Catalase Test : Well No. 6

- Scrape a loopful of growth from the surface of the 6<sup>th</sup> well. Dip the loop in a small clean test tube with 3% H<sub>2</sub>O<sub>2</sub>.
- Positive catalase test is seen as effervescence coming out from the surface of the loop. No effervescence is observed in case of negative catalase test.

**Note :** 3% H<sub>2</sub>O<sub>2</sub> solution has to be freshly prepared.

#### Nitrate Reduction : Well No. 5

- Add 1-2 drops of Sulphanilic acid (R015) and 1-2 drops of N,N-Dimethyl-1-Naphthylamine Reagent (R009).
- Immediate development of pinkish red colour on addition of reagent indicates positive reaction.
- No change in colour indicates negative reaction.

#### Voges-Proskauer's Test : Well No. 2

- Add 1-2 drops of Barritt reagent A (5%  $\alpha$ -Naphthol in absolute ethanol, R029) and 1 - 2 drops of Barritt reagent B (40% Potassium hydroxide, R030) .
- On addition of reagent pinkish red colour is observed within 10 minutes.
- No change in colour or a slight copper colour (due to reaction of Barritt reagent A and Barritt reagent B) denotes a negative reaction.

#### Carbohydrate Fermentation Test : Well No. 8 to Well No 12

- Colour of the medium changes from red colour to yellow colour due to acid production if the test is positive.
- Medium remains red in colour if the test is negative.

Identification Index of various *Bacillus* species

Tests	Malonate	Voges Proskauer's	Citrate	ONPG	Nitrate Reduction	Catalase	Arginine	Sucrose	Mannitol	Glucose	Arabinose	Trehalose
<i>B.cereus</i>	-	+	+	-	D	+	D	D	-	+	-	+
<i>B.pumilis</i>	-	+	+	+	-	+	-	+	+	+	+	+
<i>B.subtilis</i>	-	+	+	+	+	+	-	+	+	+	+	+
<i>B.coagulans</i>	-	D	-	D	-	+	D	D	-	+	D	+
<i>B.atrophaeus</i>	-	+	+	NR	+	+	NR	+	+	+	+	+
<i>B.megaterium</i>	+	-	+	+	D	+	-	+	+	+	+	+
<i>B.thuringensis</i>	NR	+	+	-	+	+	+	D	-	+	-	+

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

+ = Positive (more than 90 %)      - = Negative      NR = Not Reported      v = Variable reaction      D = Delayed reaction

## Result interpretation chart

No.	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1	Malonate	—	Malonate utilization	Bluish green	Dark Blue	Bluish green
2	Voges Proskauer's	1-2 drops of Baritt reagent A and 1-2 drops of Baritt reagent B	Detects acetoin production	Colourless/ Light yellow	Pinkish red	Colourless/ slight copper
3	Citrate	—	Citrate utilization	Light Green	Dark Blue	Light Green
4	ONPG	—	Detects Beta galactosidase	Colourless	Yellow	Colourless
5	Nitrate Reduction	1-2 drops of sulphaniilic acid and 1-2 drops of N, N-Dimethyl-1-Naphylamine	Detects Nitrate reduction	Colourless / Light yellow	Pinkish Red	Colourless
6	Catalase	3% H <sub>2</sub> O <sub>2</sub> solution	Detects Catalase activity	Colourless	Efferescence coming out from the loop	No Efferescence seen
7	Arginine	—	Arginine utilization	Olive Green to Light Purple	Purple / Dark Purple	No Change in color or yellow
8	Sucrose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
9	Mannitol	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
10	Glucose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
11	Arabinose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
12	Trehalose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink

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

1. Allow the reagents to come to room temperature after removal from the refrigerator .
2. In case of Carbohydrate fermentation test some microorganisms show weak reaction. In this case record the reaction as ± and incubate further for 24 hours. Orange colour after 72 hours of incubation should be interpreted as a negative reaction.
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. Reagents should not come in contact with skin, eyes or clothing. 3% H<sub>2</sub>O<sub>2</sub> is a extremely caustic solution, so avoid contact with skin. In case it does get on the skin, immediately flood the area with 70% Ethanol and not water, to neutralize the action.

## 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

Store between 2-8°C. Shelf-life is 12 months.



## Disclaimer :

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