

## KB009 HiCarbo™ Kit (KB009A / KB009B1 / KB009C)

### Introduction

KB009 is a comprehensive test system that can be used to study the biochemical profile of a wide variety of organisms. It can also be used for validating known laboratory strains.

A combination of 35 tests for utilization of carbohydrate tests. Kit contains Part A, Part B each having 12 carbohydrates utilization tests and Part C containing 11 sugar and 1 control

**Note** ● KB009 is available as a total kit and also individually as Part A, Part B1 and Part C

### Principle

Each HiCarbohydrate™ kit is a standardized colorimetric identification system utilizing thirty five carbohydrate utilization tests. The tests are based on the principle of pH change and substrate utilization. On incubation organisms undergo metabolic changes which are indicated by a spontaneous colour change in the media.

### Kit contents

1. Each kit contains sufficient material to perform 10 tests.
2. **10 Kits of Part A (KB009A) - Lactose, Xylose, Maltose, Fructose, Dextrose, Galactose, Raffinose, Trehalose, Melibiose, Sucrose, L-Arabinose, Mannose**
3. **10 Kits of Part B1 (KB009B1) - Inulin, Sodium gluconate, Glycerol, Salicin, Dulcitol, Inositol, Sorbitol, Mannitol, Adonitol, Arabitol, Erythritol, alpha-Methyl-D-glucoside**
4. **10 Kits of Part C (KB009C) - Rhamnose, Cellobiose, Melezitose, alpha-Methyl-D-Mannoside, Xylitol, ONPG, Esculin, D-Arabinose, Citrate, Malonate, Sorbose and 1 control**
5. Technical product insert.
6. Result Interpretation Chart and Result Entry Datasheet.

### Instructions for use

#### 1. Preparation of inoculum

- KB009 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/ M1274) or Brain Heart Infusion Agar (M211).
- Pick up a single isolated colony and inoculate in 5 ml Brain Heart Infusion Broth and incubate at 35-37°C for 4-6 hours until the inoculum turbidity is  $\geq 0.5$  OD at 620nm. Some fastidious organisms may require more than 6 hours of incubation. In this case incubate till the inoculum turbidity reaches 0.5 OD at 620nm.
- Alternatively, prepare the inoculum by picking 1-3 well isolated colonies and make a homogenous suspension in 2-3ml sterile saline. The density of the suspension should be 0.5 OD at 620nm.

**Note** ● Erroneous false negative results may be obtained if the inoculum turbidity is less than 0.5 OD.  
● Results are more prominent if an enriched culture is used instead of suspension.

#### 2. Inoculation of the kit

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

3. **Incubation** : Temperature of incubation: 35  $\pm$  2°C. Duration of incubation: 18 - 24 hours.

### Interpretation of results

Interpret results as per the result interpretation chart.

### Carbohydrate Fermentation Test :

- 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.

### ONPG Test

- Medium changes from colourless to yellow if the test is positive.
- Medium remains colourless if the test is negative.

**Esculin Hydrolysis**

- Colour of the medium changes from cream to black if the test is positive.
- Medium remains cream in colour if the test is negative.

**Citrate Utilization**

- Colour of the medium changes from yellowish green to blue if the test is positive.
- Medium remains yellowish green in colour if the test is negative.

**Malonate Utilization**

- Colour of the medium changes from light green to blue if the test is positive.
- Medium remains light green in colour if the test is negative.

## Result Interpretation chart

Test	Principle	Original colour of the medium	Positive reaction	Negative reaction
Carbohydrate utilization	Detects carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
ONPG	Detects $\beta$ -galactosidase activity	Colourless	Yellow	Colourless
Esculin hydrolysis	Detects esculin hydrolysis	Cream	Black	Cream
Citrate utilization	Detects capability of organism to utilize citrate as a sole carbon source	Green	Blue	Green
Malonate utilization	Detects capability of organism to utilize sodium malonate as a sole carbon source	Light green	Blue	Light green

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

1. In case of carbohydrate fermentation test some microorganisms show weak reaction. In this case record the reaction as  $\pm$  and incubate further for 48 hours. Orange colour after 48 hours of incubation should be interpreted as a negative reaction.
2. At times organisms give conflicting result because of mutation or the media used for isolation, cultivation and maintenance.

**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 disposal bag.

**Storage and Shelf-life**

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

**Disclaimer :**

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