

KB020 HiLacto Identification Kit

Introduction :

KB020 is identification and differentiation system of genus *Lactobacillus*. The complete list of organisms that can be identified with this system is given in the identification index provided with the kit.

Principle :

KB020 is a standardized, colorimetric identification system utilizing two conventional biochemical tests and 10 carbohydrate fermentation test. The tests are based on the principle of pH change and substrate utilization. On incubation organisms undergo 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 :

1. Each kit contains sufficient material to perform 10 tests
2. 10 strips of KB020
3. Technical product insert
4. Result Interpretation Chart and Result Entry Datasheet
5. Identification Index

Reagents required but not provided with the kit: 3% Hydrogen peroxide (H₂O₂)

Type of specimen :

Food and dairy samples, Clinical samples.

Specimen Collection and Handling :

For food, dairy or clinical samples follow appropriate techniques for handling specimens as per established guidelines.

Warning and Precautions :

In Vitro diagnostic Use only. Read the label before opening the kit. Food, dairy, 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. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in safety data sheets.

Limitations :

1. KB020 cannot be used directly on food, dairy or clinical samples. The organisms to be identified have to be first isolated and purified. Only pure cultures should be used.
2. In case of Carbohydrate fermentation test some microorganisms show weak reaction. In this case record the reaction as ± and incubate further upto 48 hours.
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.

Performance and Evaluation :

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Instructions for use Interpretation of Results :

Interpret results as per the standards given in the result interpretation chart. Addition of 3% H₂O₂ in well no 2, should be done at the end of incubation period that is after 24-48 hours with or without 5-10% CO₂.

Well No. 2 : Catalase Test:

- Scrape a loopful of growth from the surface of the well. Dip the loop in a small, clean test tube containing 3% Hydrogen peroxide.
- 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% H₂O₂ has to be freshly prepared

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 .

Disposal of used material :

After use, kits and the materials 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.

Instructions for use

Preparation of inoculum :

- Isolate the organism to be identified on a medium like Lactobacillus MRS Agar (M641) or Soyabean Casein Digest Agar (M290).
- Pick up a single isolated colony and inoculate in 5 ml Lactobacillus MRS Broth (M369) and incubate either at 35-37°C or 35-37°C with 5-10 % CO₂ as per organism's requirement for 6-18 hours until the inoculum turbidity is 0.10D at 620 nm or 0.5 McFarland standards.
- Alternatively, prepare the inoculum by picking 1-3 well isolated colonies and make a homogenous suspension in 2-3 ml sterile saline. The density of the suspension should be 0.10D at 620nm or 0.5 McFarland standards.

Note : Erroneous false negative results may be obtained if the inoculum turbidity is less than 0.1 OD.
Results are more prominent if an enriched culture is used instead of suspension.

Inoculation of the kit :

- Open the kit aseptically. Peel off the sealing tape.
- Inoculate each well with 50 µl of the above inoculum by surface inoculation method.
- Alternatively the kit can be inoculated by stabbing each individual well with a loopful of inoculum.

Incubation :

- Temperature of incubation : 35 - 37°C with or without 5-10% CO₂. Duration of incubation : 24-48 hours.

Storage and Shelf life :

On receipt, store at 2-8°C. Shelf life is 12 months. Product performance is best if used within stated expiry period.

Biochemical reactions of KB020

Well No	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1	Esculin Hydrolysis	-	Detects Hydrolysis of Esculin	Cream / Yellow	Black	Cream / Yellow
2	Catalase	3% H ₂ O ₂	Detects Catalase activity	Colours	Effervescence coming out from the loop	No effervescence seen
3	Xylose	-	Fermentation of Xylose	Purple	Yellow	Purple
4	Cellobiose	-	Fermentation of Cellobiose	Purple	Yellow	Purple
5	Arabinose	-	Fermentation of Arabinose	Purple	Yellow	Purple
6	Maltose	-	Fermentation of Maltose	Purple	Yellow	Purple
7	Galactose	-	Fermentation of Galactose	Purple	Yellow	Purple
8	Mannose	-	Fermentation of Mannose	Purple	Yellow	Purple
9	Melibiose	-	Fermentation of Melibiose	Purple	Yellow	Purple
10	Raffinose	-	Fermentation of Raffinose	Purple	Yellow	Purple
11	Sucrose	-	Fermentation of Sucrose	Purple	Yellow	Purple
12	Trehalose	-	Fermentation of Trehalose	Purple	Yellow	Purple

	Esculin	Catalase	Xylose	Cellobiose	Arabinose	Maltose	Galactose	Mannose	Melibiose	Raffinose	Sucrose	Trehalose
<i>L. acidifarinae</i>	N	N	N	N	P	P	P	N	N	N	N	N
<i>L. antri</i>	N	N	D	N	P	P	P	N	P	P	P	N
<i>L. brevis</i>	D	N	D	N	P	P	D	N	P	D	D	N
<i>L. buchneri</i>	D	N	D	N	P	P	D	N	P	D	D	N
<i>L. colemominis</i>	N	N	N	N	N	D	N	N	N	N	N	N
<i>L. collinoides</i>	P	N	P	N	P	P	P	N	P	N	N	N
<i>L. diolivorans</i>	N	N	P	N	P	P	P	N	P	N	N	N
<i>L. durianis</i>	D	N	P	N	P	N	D	N	N	N	N	N
<i>L. ferintoshensis</i>	P	N	P	D	P	P	P	P	D	D	P	P
<i>L. fermentum</i>	N	N	D	D	D	P	P	N	P	P	P	D
<i>L. fructivorans</i>	N	N	N	N	N	D	N	N	N	N	D	N
<i>L. frumenti</i>	P	N	N	P	D	P	P	P	P	P	P	P
<i>L. gastricus</i>	N	N	N	P	D	P	P	P	P	P	P	P
<i>L. hammesii</i>	N	N	P	P	P	P	P	P	N	N	N	P
<i>L. hilgardii</i>	N	N	P	N	N	P	D	N	N	N	D	N
<i>L. ingluviei</i>	D	N	P	N	P	D	N	D	N	D	P	N
<i>L. kefir</i>	N	N	N	N	D	P	N	N	P	N	N	N
<i>L. kunkeei</i>	N	N	N	N	ND	N	N	N	N	N	P	N
<i>L. lindneri</i>	N	N	N	N	N	P	N	N	N	N	N	N
<i>L. malefermentans</i>	N	N	V	N	N	P	N	N	N	N	N	N
<i>L. mucosae</i>	P	N	P	N	D	P	D	N	D	D	P	N
<i>L. oris</i>	D	N	P	D	P	P	P	D	P	P	P	D
<i>L. panis</i>	P	N	P	N	P	P	P	P	P	P	P	N
<i>L. parabuchneri</i>	N	N	N	N	P	P	P	ND	P	P	P	N
<i>L. paracollinoides</i>	ND	N	P	N	D	P	ND	N	P	N	N	N
<i>L. parakefir</i>	N	N	N	N	P	P	P	N	D	N	N	N
<i>L. pontis</i>	N	N	N	N	N	P	D	N	D	D	P	ND
<i>L. reuteri</i>	ND	N	N	N	P	P	P	N	P	P	P	N
<i>L. rossiae</i>	N	N	D	N	P	P	D	D	D	N	N	N
<i>L. sanfranciscensis</i>	ND	N	N	N	N	P	D	N	N	N	D	N
<i>L. spicheri</i>	N	N	P	N	N	P	N	N	N	N	N	N
<i>L. suebicus</i>	N	N	P	D	P	P	P	ND	D	N	D	N
<i>L. thermotolerans</i>	P	N	P	ND	P	ND	N	ND	D	D	ND	ND
<i>L. vaccinostercus</i>	N	N	P	N	P	P	N	N	N	N	N	N
<i>L. vaginalis</i>	D	N	N	N	N	P	P	P	P	P	P	N
<i>L. zymae</i>	N	N	D	N	P	P	D	N	N	N	N	N

key : P : Positive, N : Negative, ND : Not detected, D : Variable

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