# bKIT *Lactobacillus acidophilus* HA-122

Real-Time PCR assay

**Code: bKTPR-LAHA122.01**


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## Introduction

*Lactobacillus* classification trace back to 1901, when, based on biochemical and morphological characteristics, Beijerinck ([1]) M.W proposed the genus. Using the same approach Moro ([2]), Hansen and Mocquot ([3]) proposed the species *Lactobacillus acidophilus*. Nowadays, many efforts focus on the correlation between taxonomic classification with traditional procedures and DNA molecular methods. Consistently with this trend, traditional culture approaches are increasingly assisted by DNA molecular methods ([4]). Among these, Real-Time PCR emerged for its sensitivity, rapidity, reliability, specificity and repeatability making it a well-established method for the detection, quantification, and typing of different microbial agents in the areas of clinical and veterinary diagnostics and food safety ([5]).

([1]) BEIJERINCK (M.W.): Sur les ferments lactiques de l'industrie. Archives Néerlandaises des Sciences Exactes et Naturelles (Section 2), 1901, 6, 212-243.
([2]) MORO (E.): Über den Bacillus acidophilus n. sp. Jahrbuch fur Kinderheilkunde und physiche Erziehung, 1900, 52, 38-55.
([3]) HANSEN (P.A.) and MOCQUOT (G.): Lactobacillus acidophilus (Moro) comb. nov. International Journal of Systematic Bacteriology, 1970, 20, 325-327.
([4]) Mianzhi Y, Shah NP. Contemporary nucleic acid-based molecular techniques for detection, identification, and characterization of Bifidobacterium. Crit Rev Food Sci Nutr. 2017 Mar 24;57(5):987-1016. doi: 10.1080/10408398.2015.1023761. Review.PubMed PMID: 26565761.
([5]) Kralik P, Ricchi M. A Basic Guide to Real Time PCR in Microbial Diagnostics: Definitions, Parameters, and Everything. Front Microbiol. 2017 Feb 2;8:108. doi: 10.3389/fmicb.2017.00108. eCollection 2017. Review. PubMed PMID: 28210243; PubMed Central PMCID: PMC5288344.

## Principle

SYBR® Green Real-Time PCR (qPCR) assay for the detection of *Lactobacillus acidophilus* HA-122. The product is intended for research purpose only.

## NHPRA validation

In the validation trials performed by NHPRA (Natural Health Product Research Alliance) the following strains were tested: *Lactobacillus acidophilus HA-122*, *Lactobacillus casei HA-108*, *Lactobacillus paracasei HA-196*, *Lactobacillus paracasei HA-274*, *Lactobacillus acidophilus R0418*.
Moreover, assay performances were assessed in mixtures containing the DNA of the strains listed above. All DNA solutions tested were normalized to the concentration of 1 ng/µL before use. All target and non-target DNA sample solutions were successfully classified. For more details, contact us at support@hyris.net.

## bKIT *Lactobacillus acidophilus* HA-122 packaging

**Part Number: bKTPR-LAHA122.01 -50**

| | |
|---|---|
| qPCR Master Mix (1 tube) | 50 tests |
| Positive Control (1 tube) | 10 tests |
| Negative Control (1 tube) | 10 tests |

**Part Number: bKTPR-LAHA122.01 -100**

| | |
|---|---|
| qPCR Master Mix (2 tubes) | 2 x 50 tests |
| Positive Control (1 tube) | 20 tests |
| Negative Control (1 tube) | 20 tests |

## Storage

-20°C. Avoid prolonged exposure to light and repeated freeze and thaw cycles.

## Shelf life

If the bKIT is correctly stored, at constant-temperature freezer, its performance is guaranteed until the shelf life indicated on the tubes.

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## Additional material/reagents required

- DNA extraction tools and reagents.
- Nuclease-free water.
- Gloves.
- Pipettes.
- bCUBE® instrument or other Real-Time PCR instrument (*) with filters calibrated for SYBR® Green.
- bCUBE® sample loading cartridge or, if using other Real-Time PCR instrument, samples loading support according to the instrument specifications.

*(*) This assay was especially developed to be used in association with the bCUBE® instrument, available from Hyris Ltd, but can be used also with any other compatible thermal cycler.*

## DNA extraction

In the validation trials performed by NHPRA the DNA was extracted using NucleoSpin® Food (MACHEREY-NAGEL) and normalized to the concentration of 1 ng/µL. For more details, contact us at support@hyris.net.

## Reaction set-up

a. Thaw all the bKIT components by placing the tubes on ice.
b. Gently mix the tubes content by swirling the tubes.
c. Spin the tubes to let the content down.
d. In new tubes, one for each sample, including the Negative Control and the Positive Control of the bKIT, prepare the Reaction Mix as shown in the table below:

| Components | Volume |
|---|---|
| DNA sample (normalized to the concentration of 1 ng/µL) or Positive Control or Negative Control | 2 µL |
| qPCR Mastermix | 18 µL |
| **Total Volume** | **20 µL** |

## Cartridge set-up

The procedure described is for the bCUBE® cartridge, but, if using a different Real-Time PCR instrument, the same procedure can be adopted for other loading sample supports with minor modifications.

**1. Samples set-up**

Samples of the following types must be prepared to be loaded on the cartridge:
Positive Control for *Lactobacillus acidophilus* HA-122.
Negative Control for *Lactobacillus acidophilus* HA-122.
Sample(s) to be tested.

**2. Cartridge Loading**

a. Load the sample prepared as described in the previous section.
b. Carefully seal the cartridge with adhesive film in order to avoid any contamination.
c. Load the cartridge onto the bCUBE®, then start the run.

## Method set-up

Set up the run method using the following conditions, depending on the instrument you use.

**1. On the bCUBE®**

a. Login on the bAPP.
b. Set-up "New Analysis" and Select the "Lactobacillus acidophilus HA-122 1.x" from the "Global recipes" list.
c. Specify the "Well types" for each of the loaded sample as follows (**Fig. 1**):

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"PosCtrl" for the well loaded with *Lactobacillus acidophilus* HA-122 Positive Control.
"NegCtrl" for the well loaded with *Lactobacillus acidophilus* HA-122 Negative Control.
"Sample" for the wells loaded with samples under analysis.


**Fig 1. Cartridge set-up**
An example of cartridge set-up on the bAPP for one replicate of a sample to be analyzed is shown.

**2. <u>On a compatible Real-Time PCR instrument</u>**
Please, contact us for the protocol set-up on the instrument.

## Reading the results

**1. <u>On the bCUBE®</u>**

a. The presence of the target *Lactobacillus acidophilus* HA-122 in the **Positive Control** or in the **sample** under analysis will generate an amplification curve **(Fig. 2a)** and a melting curve with a specific melting peak **(Fig. 2b)**.

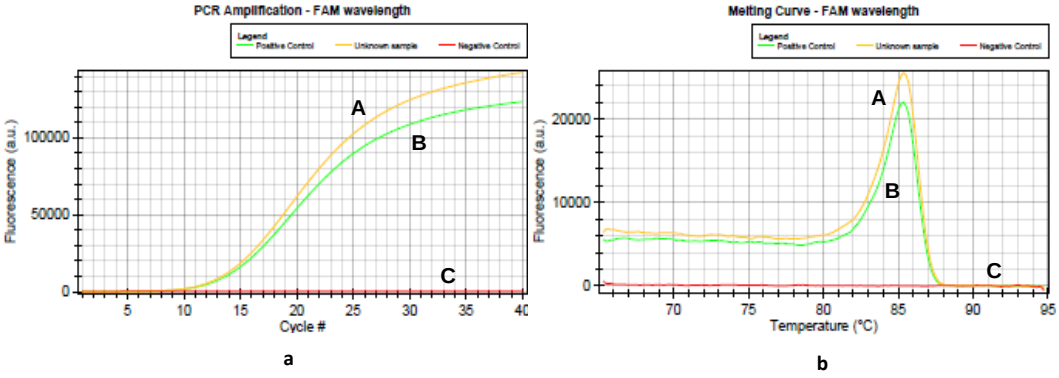


**Fig.2. Amplification and melting plots**
In the plots, the amplification curve (**Fig. 2a**) and the specific melting peak (**Fig. 2b**) of a *Lactobacillus acidophilus* HA-122 containing **sample** (**A**), the **Positive Control** (**B**), and the **Negative Control** (**C**) are shown.

b. At the end of analysis each well will be labelled depending on the "Well type" as described in the table below and samples classification will be shown on the pdf report of the analysis (**Fig. 3**).

| Well type | Possible labels | Label meaning |
|---|---|---|
| **Positive Control (PosCtrl)** | **OK** | Amplification curve and specific melting peak present |
| | **KO** | Amplification curve and or specific melting peak absent |

| Well type | Possible labels | Label meaning |
|---|---|---|
| **Negative Control (NegCtrl)** | **OK** | Amplification curve and specific melting peak absent |
| | **KO** | Amplification curve and or specific melting peak present |

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| Well type | Possible labels | Label meaning |
|---|---|---|
| Sample | Present | *Lactobacillus acidophilus* HA-122 is present in the sample |
| | Absent | *Lactobacillus acidophilus* HA-122 is absent from the sample |
| | Indeterminate | The test is not conclusive and should be repeated (**) |

*(**) If the "Indeterminate" classification persists, contact us at support@hyris.net.*

| Results for target *Lactobacillus acidophilus HA-122* | |
|---|---|
| Positive control (PosCtrl) | OK |
| Unknown sample (Sample) | Present |
| Negative control (NegCtrl) | OK |

**Fig.3. Analysis results table**
An example of the results table, as reported in the pdf report of the analysis, is shown.

**2. On a compatible Real-Time PCR instrument**
Please, contact us for results interpretation.

## Troubleshooting

**1. Results show no amplification, or anomalous amplification curves**

| Possible causes | Corrective actions |
|---|---|
| Evaporation of the sample due to inadequate sealing of the plate/strips | Repeat the test using the appropriate materials and tools to seal correctly the plate/strips |
| Consumables are not appropriate for the method | Repeat the test using consumables recommended by the supplier of the Real-Time PCR instrument |
| The quality of nucleic acid extracted is low | Repeat the extraction step. Ensure that the method of extraction has been performed correctly. In any doubt, contact us at support@hyris.net. |

**2. No amplification curve is observed for the Positive Control**

| Possible causes | Corrective actions |
|---|---|
| The Positive Control provided with the assay was not added into the reaction well | Repeat the test adding the Positive Control. If the problem persists, contact us at support@hyris.net. |

**3. An amplification curve with a specific melting peak is observed for the Negative Control**

| Possible causes | Corrective actions |
|---|---|
| Contamination of the Negative Control or the qPCR Master Mix with target-positive DNA | Repeat the test by applying appropriate quality procedures to prevent contamination. Correctly seal the cartridge or plate/strips. If the problem persists, contact us at support@hyris.net. |

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