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# **bKIT *Bifidobacterium infantis* HA-116**

Real-Time PCR assay

Code: bKTPR-BIHA116.01

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## **Hyris Ltd**

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### **Hyris Headquarters**

Lower Ground Floor, One George Yard,  
EC3V 9DF, London UK  
Phone: +44.2036082968  
Mail: office@hyris.net

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### **Hyris Research Center**

Corso Garibaldi, 60  
20121 Milano, Italy  
Phone: +39.02.82951302  
Mail: administrator@hyris.net

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### **Hyris Asia Pac**

38 Ang Mo Kio Industrial Park 2 #02-07A  
569511 Singapore  
Phone: +65.8160.7207  
Mail: office@hyris.net

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[www.hyris.net](http://www.hyris.net)

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**Introduction**

Current *Bifidobacterium* classification trace back to 1924, when Orla-Jensen <sup>(1)</sup> suggested its separation from the genus *Lactobacillus*. Later, in 1963, Reuter <sup>(2)</sup> started the characterization of the genus based on biochemical characteristics proposing eight species, including *Bifidobacterium infantis*. 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 <sup>(3)</sup>. 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 <sup>(4)</sup>.

<sup>(1)</sup> ORLA-JENSEN (S.): Classification des bactéries lactiques. Lait, 1924, 4, 468-474.

<sup>(2)</sup> REUTER (G.): Vergleichenden Untersuchung über die Bifidus-Flora im Säuglings und Erwachsenenstuhl. Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. Abteilung I, 1963, 191, 486-507.

<sup>(3)</sup> 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.

<sup>(4)</sup> 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 *Bifidobacterium infantis* HA-116. 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: *Bifidobacterium breve* HA-129, *Bifidobacterium bifidum* HA-132, *Bifidobacterium longum* HA-135, *Bifidobacterium infantis* HA-116 and *Bifidobacterium longum* RO-175. 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](mailto:support@hyris.net).

**bKIT *Bifidobacterium infantis* HA-116 packaging**
**Part Number: bKTPR-BIHA116.01 -50**

qPCR Master Mix (1 tube, blue cap)	50 tests
Positive Control (1 tube, green cap)	10 tests
Negative Control (1 tube, red cap)	10 tests

**Part Number: bKTPR-BIHA116.01 -100**

qPCR Master Mix (2 tubes, blue cap)	2 x 50 tests
Positive Control (1 tube, green cap)	20 tests
Negative Control (1 tube, red cap)	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.

**Additional material/reagents required**

- DNA extraction tools and reagents.

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- 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](mailto: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 <b>Positive Control</b> or <b>Negative Control</b>	2 μL
qPCR Mastermix	18 μL
<b>Total Volume</b>	<b>20 μL</b>

**Cartridge set-up**

The procedure described is for the bCUBE® cartridge, but, if using a different Real-Time PCR, 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 *Bifidobacterium infantis* HA-116.

**Negative Control** for *Bifidobacterium infantis* HA-116.

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 “*Bifidobacterium infantis* HA-116 1.x” from the “Global recipes” list.
- c. Specify the “Well types” for each of the loaded sample as follows (**Fig. 1**): “PosCtrl” for the well loaded with *Bifidobacterium infantis* HA-116 **Positive Control**.

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“NegCtrl” for the well loaded with *Bifidobacterium infantis* HA-116 **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.

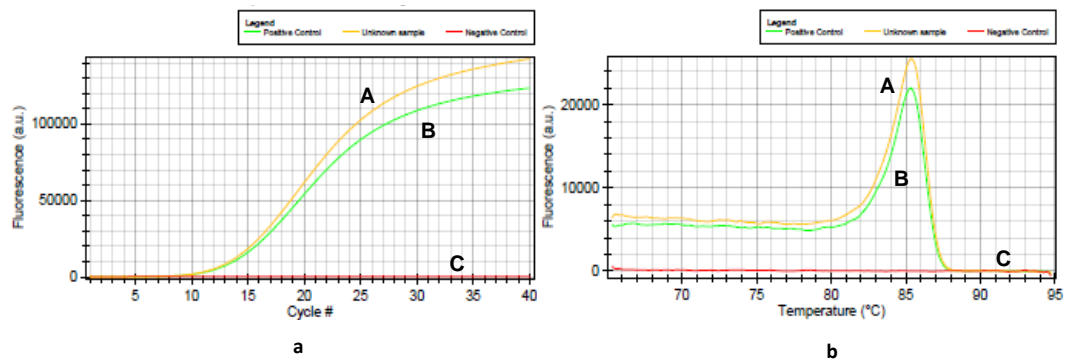
**2. On a compatible Real-Time PCR instrument**

Please, contact us for the protocol set-up on the instrument.

**Reading the results**

**1. On the bCUBE®**

- a. The presence of the target *Bifidobacterium infantis* HA-116 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 *Bifidobacterium infantis* HA-116 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	The positive control behavior is within the expected range.
	KO	The positive control behavior isn't within the expected range. Please see <b>Troubleshooting</b> section below.
Negative Control (NegCtrl)	OK	The negative control behavior is within the expected range.
	KO	The negative control behavior isn't within the expected range. Please see <b>Troubleshooting</b> section below

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Well type	Possible labels	Label meaning
Sample	Present	The target DNA sequence, characteristic of <i>Bifidobacterium infantis</i> HA-116, is present in the sample**.
	Absent	The target DNA sequence, characteristic of <i>Bifidobacterium infantis</i> HA-116, is absent from the sample** or in amount below the limit of detection of the assay.
	Indeterminate	The test is not conclusive and should be repeated. If the "Indeterminate" classification persists, contact us at <a href="mailto:support@hyris.net">support@hyris.net</a> .

(\*\*) The assay has been designed to discriminate DNA polymorphisms between target and non-target sequences; nevertheless, correct label classification applies and can be ensured only with validated processing conditions, including samples and matrixes tested during the validation of the assay.

Results for target <i>Bifidobacterium infantis</i> HA-116	
Positive control (PosCtrl)	OK
Negative control (NegCtrl)	OK
Unknown sample (Sample)	Present

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 <a href="mailto:support@hyris.net">support@hyris.net</a> .

**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 <a href="mailto:support@hyris.net">support@hyris.net</a> .
Some issues with reaction components and/or reaction conditions occurred	Repeat the experiment checking that all step required for the analysis have been performed correctly. If the problem persists, contact us at <a href="mailto:support@hyris.net">support@hyris.net</a> .

**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 <a href="mailto:support@hyris.net">support@hyris.net</a> .

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