



NHP  
RESEARCH  
ALLIANCE



[bapp.hyris.net/#/bkits](http://bapp.hyris.net/#/bkits)

# **bKIT *Lactobacillus reuteri* HA-188**

**PN bKTPR-LRHA188.01**

**Real-Time PCR assay**

## **Hyris Ltd**

### **Hyris Headquarters**

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

### **Hyris Research Center**

Corso Garibaldi, 60  
20121 Milano, Italy  
Phone: +39.02.82951302  
Mail: [administrator@hyris.net](mailto:administrator@hyris.net)

### **Hyris Asia Pac**

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

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

## Hyris Ltd

### Hyris Headquarters

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

### Hyris Research Center

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

### Hyris Asia Pac

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

www.hyris.net

## WARNING AND PRECAUTIONS

Please check Kits integrity before use. Use of deteriorated Kits may cause lack of results and/or equivocal results. Do not mix the components of kits with different batch number. Do not interchange reagents with other kits, or components from different lots. Do not use reagents after their expiration date. This kit is designed to be used by personnel trained to follow correct molecular biology precautions.

## WARRANTY AND RESPONSIBILITIES

Hyris Ltd guarantees the buyer exclusively concerning the quality of reagents and of the components used to produce the Kits. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits our responsibility to the replacement of the product. No other warranties, of any kind, express or implied, including, without limitation, implicit warranties of commercialisation ability or adequacy for a given purpose, are provided by Hyris Ltd. Hyris Ltd is not responsible and cannot anyway be considered responsible or jointly responsible for possible direct and indirect damages resulting from the utilization of the Kits by the user. The user consciously and under his/her own responsibilities decides for the utilization purposes of the Kits and uses it the way he/she considers most suitable in order to reach his/her goals and/or objectives. Hyris Ltd will not be held responsible for any direct, indirect, consequential or incidental damage resulting of the use, misuses, results of the use or inability to use any product. Some of the applications which may be performed with this product may be covered by applicable patents in certain countries. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application. Hyris Ltd does not encourage the unlicensed use of patented applications. The Kits may require the use of Taq Polymerase enzyme, DNA binding components and fluorochromes/quencher, often registered as trademark by companies. TaqMan® is a trademark of Roche Molecular Systems, Inc. FAM™, HEX™ and ROX™ are a trademark of Thermo Fisher Scientific or its subsidiaries. SYBR is a registered trademark of Molecular Probes, Inc. (Thermo Fisher Scientific). MGB probes are a trademark of Elitech group. The Kits have been internally tested by our quality control. Any responsibility is waived if the warranty of quality control does not refer to the specific Kits. The user is personally responsible for data that he/she will obtain and/or he/she will supply to third parties using these kits. Once the sealed package is opened the user accepts all the conditions without fail; if the package is still sealed the kit can be returned and the user can be refunded. Kits components are intended, developed, designed, and sold for Research Purpose Only. Product claims are subject to change. Therefore, please refer to our website (www.hyris.net) for the most up-to-date information on Hyris Ltd products.

## Introduction

*Lactobacillus* classification trace back to 1901, when, based on biochemical and morphological characteristics, Beijerinck <sup>(1)</sup> M.W proposed the genus. Using the same approach Kandler et al. <sup>(2)</sup> proposed the species *Lactobacillus reuteri*. 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> BEIJERINCK (M.W.): Sur les ferments lactiques de l'industrie. Archives Néerlandaises des Sciences Exactes et Naturelles (Section 2), 1901, 6, 212-243.

<sup>(2)</sup> KANDLER (O.), STETTER (K.O.) and KÖHL (R.): *Lactobacillus reuteri* sp. nov., a new species of heterofermentative lactobacilli. Zentralbl. Mikrobiol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig., 1980, C1, 264-269.

<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 *Lactobacillus reuteri* HA-188. The product is intended for research purpose only.

## NHPRA validation

The validation was performed on the bCUBE platform following the methods and the guidelines accepted and published on [J AOAC Int. 2019 Nov 1;102\(6\):1774-1778](#).

In the validation trials performed by NHPRA (Natural Health Product Research Alliance) the following strains were tested: *Lactobacillus reuteri* HA-188, *Lactobacillus acidophilus* HA-122, *Lactobacillus acidophilus* R0418, *Lactobacillus plantarum* HA-119, *Lactobacillus delbrueckii subsp. bulgaricus* Lb-87, *Lactobacillus delbrueckii subsp. bulgaricus* HA-137, *Lactobacillus helveticus* R0052.

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 *Lactobacillus reuteri* HA-188 packaging

### Part number: bKTPR-LRHA188.01-50

qPCR Master Mix (1 tube, blue cap)	50 tests
Positive Control (1 tube, green cap)	14 tests
Negative Control (1 tube, red cap)	14 tests

### Part number: bKTPR-LRHA188.01-100

qPCR Master Mix (2 tubes, blue cap)	2 x 50 tests
Positive Control (1 tube, green cap)	28 tests
Negative Control (1 tube, red cap)	28 tests

## Storage

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

## Shelf life

## Hyris Ltd

### Hyris Headquarters

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

### Hyris Research Center

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

### Hyris Asia Pac

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

www.hyris.net

## WARNING AND PRECAUTIONS

Please check Kits integrity before use. Use of deteriorated Kits may cause lack of results and/or equivocal results. Do not mix the components of kits with different batch number. Do not interchange reagents with other kits, or components from different lots. Do not use reagents after their expiration date. This kit is designed to be used by personnel trained to follow correct molecular biology precautions.

## WARRANTY AND RESPONSIBILITIES

Hyris Ltd guarantees the buyer exclusively concerning the quality of reagents and of the components used to produce the Kits. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits our responsibility to the replacement of the product. No other warranties, of any kind, express or implied, including, without limitation, implicit warranties of commercialisation ability or adequacy for a given purpose, are provided by Hyris Ltd. Hyris Ltd is not responsible and cannot anyway be considered responsible or jointly responsible for possible direct and indirect damages resulting from the utilization of the Kits by the user. The user consciously and under his/her own responsibilities decides for the utilization purposes of the Kits and uses it the way he/she considers most suitable in order to reach his/her goals and/or objectives. Hyris Ltd will not be held responsible for any direct, indirect, consequential or incidental damage resulting of the use, misuses, results of the use or inability to use any product. Some of the applications which may be performed with this product may be covered by applicable patents in certain countries. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application. Hyris Ltd does not encourage the unlicensed use of patented applications. The Kits may require the use of Taq Polymerase enzyme, DNA binding components and fluorochromes/quencher, often registered as trademark by companies. TaqMan® is a trademark of Roche Molecular Systems, Inc. FAM™, HEX™ and ROX™ are a trademark of Thermo Fisher Scientific or its subsidiaries. SYBR is a registered trademark of Molecular Probes, Inc. (Thermo Fisher Scientific). MGB probes are a trademark of Elitech group. The Kits have been internally tested by our quality control. Any responsibility is waived if the warranty of quality control does not refer to the specific Kits. The user is personally responsible for data that he/she will obtain and/or he/she will supply to third parties using these kits. Once the sealed package is opened the user accepts all the conditions without fail; if the package is still sealed the kit can be returned and the user can be refunded. Kits components are intended, developed, designed, and sold for Research Purpose Only. Product claims are subject to change. Therefore, please refer to our website (www.hyris.net) for the most up-to-date information on Hyris Ltd products.

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.
- 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 cyclers.

## DNA extraction

The assay has been validated on DNA samples obtained through the application of one or more protocols that are available for download on the bAPP.

The procedure for the download is the following:

- Login on the bAPP.
- Go on bKITs section and select **bKTPR-LRHA188.01**.
- Click the button "Download DNA extraction method".

## Reaction set-up

- Thaw all the bKIT components by placing the tubes on ice.
- Gently mix the tubes content by swirling the tubes.
- Spin the tubes to let the content down.
- 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 <b>1 ng/μL</b> ) 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 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 reuteri* HA-188.

**Negative Control** for *Lactobacillus reuteri* HA-188.

Sample(s) to be tested.

### 2. Cartridge Loading

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

## Hyris Ltd

### Hyris Headquarters

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

### Hyris Research Center

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

### Hyris Asia Pac

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

www.hyris.net

## WARNING AND PRECAUTIONS

Please check Kits integrity before use. Use of deteriorated Kits may cause lack of results and/or equivocal results. Do not mix the components of kits with different batch number. Do not interchange reagents with other kits, or components from different lots. Do not use reagents after their expiration date. This kit is designed to be used by personnel trained to follow correct molecular biology precautions.

## WARRANTY AND RESPONSIBILITIES

Hyris Ltd guarantees the buyer exclusively concerning the quality of reagents and of the components used to produce the Kits. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits our responsibility to the replacement of the product. No other warranties, of any kind, express or implied, including, without limitation, implicit warranties of commercialisation ability or adequacy for a given purpose, are provided by Hyris Ltd. Hyris Ltd is not responsible and cannot anyway be considered responsible or jointly responsible for possible direct and indirect damages resulting from the utilization of the Kits by the user. The user consciously and under his/her own responsibilities decides for the utilization purposes of the Kits and uses it the way he/she considers most suitable in order to reach his/her goals and/or objectives. Hyris Ltd will not be held responsible for any direct, indirect, consequential or incidental damage resulting of the use, misuses, results of the use or inability to use any product. Some of the applications which may be performed with this product may be covered by applicable patents in certain countries. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application. Hyris Ltd does not encourage the unlicensed use of patented applications. The Kits may require the use of Taq Polymerase enzyme, DNA binding components and fluorochromes/quencher, often registered as trademark by companies. TaqMan® is a trademark of Roche Molecular Systems, Inc. FAM™, HEX™ and ROX™ are a trademark of Thermo Fisher Scientific or its subsidiaries. SYBR is a registered trademark of Molecular Probes, Inc. (Thermo Fisher Scientific). MGB probes are a trademark of Elitech group. The Kits have been internally tested by our quality control. Any responsibility is waived if the warranty of quality control does not refer to the specific Kits. The user is personally responsible for data that he/she will obtain and/or he/she will supply to third parties using these kits. Once the sealed package is opened the user accepts all the conditions without fail; if the package is still sealed the kit can be returned and the user can be refunded. Kits components are intended, developed, designed, and sold for Research Purpose Only. Product claims are subject to change. Therefore, please refer to our website (www.hyris.net) for the most up-to-date information on Hyris Ltd products.

## Analysis set-up

Set up the run method using the proper procedure, depending on the instrument you use.

### 1. On the bCUBE®

- Login on the bAPP/bPANEL.
  - If you are using the **bAPP**, set-up “New Analysis”, flag bKIT, and select “**Probiotics**” → “**bKITPR-LRHA188.01**”; alternatively, scan the QRCode on the front page of the present document, or on the bKIT packaging.
  - If you are using the **bPANEL**, set-up “New Analysis” and select “**Lactobacillus reuteri HA-188 1.x**” (x indicates the number of the last version available) from the “Global recipes” list.
- Specify the “Well types” for each of the loaded sample as follows (**Fig. 1**):
  - “PosCtrl” for the well loaded with *Lactobacillus reuteri* HA-188. **Positive Control**.
  - “NegCtrl” for the well loaded with *Lactobacillus reuteri* HA-188. **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.

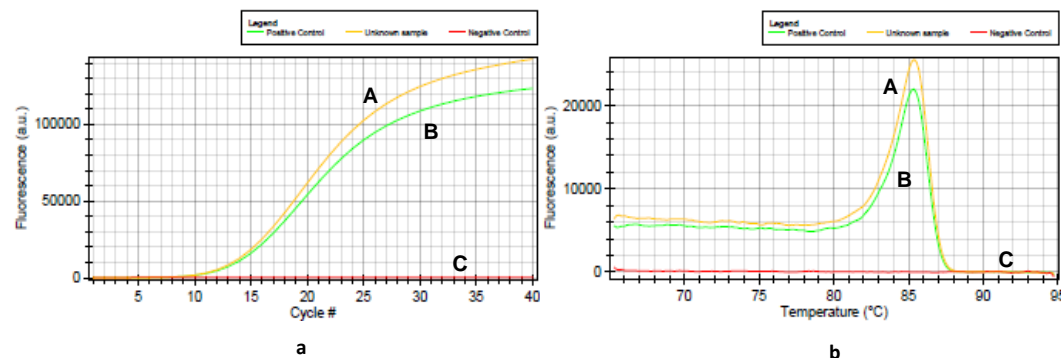
### 2. On a compatible Real-Time PCR instrument

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

## Reading the results

### 1. On the bCUBE®

- The presence of the target *Lactobacillus reuteri* HA-188 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 reuteri* HA-188 containing **sample (A)**, the **Positive Control (B)**, and the **Negative Control (C)** are shown.

## Hyris Ltd

### Hyris Headquarters

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

### Hyris Research Center

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

### Hyris Asia Pac

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

www.hyris.net

## WARNING AND PRECAUTIONS

Please check Kits integrity before use. Use of deteriorated Kits may cause lack of results and/or equivocal results. Do not mix the components of kits with different batch number. Do not interchange reagents with other kits, or components from different lots. Do not use reagents after their expiration date. This kit is designed to be used by personnel trained to follow correct molecular biology precautions.

## WARRANTY AND RESPONSIBILITIES

Hyris Ltd guarantees the buyer exclusively concerning the quality of reagents and of the components used to produce the Kits. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits our responsibility to the replacement of the product. No other warranties, of any kind, express or implied, including, without limitation, implicit warranties of commercialisation ability or adequacy for a given purpose, are provided by Hyris Ltd. Hyris Ltd is not responsible and cannot anyway be considered responsible or jointly responsible for possible direct and indirect damages resulting from the utilization of the Kits by the user. The user consciously and under his/her own responsibilities decides for the utilization purposes of the Kits and uses it the way he/she considers most suitable in order to reach his/her goals and/or objectives. Hyris Ltd will not be held responsible for any direct, indirect, consequential or incidental damage resulting of the use, misuses, results of the use or inability to use any product. Some of the applications which may be performed with this product may be covered by applicable patents in certain countries. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application. Hyris Ltd does not encourage the unlicensed use of patented applications. The Kits may require the use of Taq Polymerase enzyme, DNA binding components and fluorochromes/quencher, often registered as trademark by companies. TaqMan® is a trademark of Roche Molecular Systems, Inc. FAM™, HEX™ and ROX™ are a trademark of Thermo Fisher Scientific or its subsidiaries. SYBR is a registered trademark of Molecular Probes, Inc. (Thermo Fisher Scientific). MGB probes are a trademark of Elitech group. The Kits have been internally tested by our quality control. Any responsibility is waived if the warranty of quality control does not refer to the specific Kits. The user is personally responsible for data that he/she will obtain and/or he/she will supply to third parties using these kits. Once the sealed package is opened the user accepts all the conditions without fail; if the package is still sealed the kit can be returned and the user can be refunded. Kits components are intended, developed, designed, and sold for Research Purpose Only. Product claims are subject to change. Therefore, please refer to our website (www.hyris.net) for the most up-to-date information on Hyris Ltd products.

- 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.

Well type	Possible labels	Label meaning
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

Well type	Possible labels	Label meaning
Sample	Present	The target DNA sequence, characteristic of <i>Lactobacillus reuteri</i> HA-188, is present in the sample**.
	Absent	The target DNA sequence, characteristic of <i>Lactobacillus reuteri</i> HA-188, 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>Lactobacillus reuteri</i> HA-188		
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

## Hyris Ltd

### Hyris Headquarters

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

### Hyris Research Center

Corso Garibaldi, 60  
 20121 Milano, Italy  
 Phone: +39.02.82951302  
 Mail: [administrator@hyris.net](mailto:administrator@hyris.net)

### Hyris Asia Pac

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

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

## WARNING AND PRECAUTIONS

Please check Kits integrity before use. Use of deteriorated Kits may cause lack of results and/or equivocal results. Do not mix the components of kits with different batch number. Do not interchange reagents with other kits, or components from different lots. Do not use reagents after their expiration date. This kit is designed to be used by personnel trained to follow correct molecular biology precautions.

## WARRANTY AND RESPONSIBILITIES

Hyris Ltd guarantees the buyer exclusively concerning the quality of reagents and of the components used to produce the Kits. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits our responsibility to the replacement of the product. No other warranties, of any kind, express or implied, including, without limitation, implicit warranties of commercialisation ability or adequacy for a given purpose, are provided by Hyris Ltd. Hyris Ltd is not responsible and cannot anyway be considered responsible or jointly responsible for possible direct and indirect damages resulting from the utilization of the Kits by the user. The user consciously and under his/her own responsibilities decides for the utilization purposes of the Kits and uses it the way he/she considers most suitable in order to reach his/her goals and/or objectives. Hyris Ltd will not be held responsible for any direct, indirect, consequential or incidental damage resulting of the use, misuses, results of the use or inability to use any product. Some of the applications which may be performed with this product may be covered by applicable patents in certain countries. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application. Hyris Ltd does not encourage the unlicensed use of patented applications.

The Kits may require the use of Taq Polymerase enzyme, DNA binding components and fluorochromes/quencher, often registered as trademark by companies. TaqMan® is a trademark of Roche Molecular Systems, Inc. FAM™, HEX™ and ROX™ are a trademark of Thermo Fisher Scientific or its subsidiaries. SYBR is a registered trademark of Molecular Probes, Inc. (Thermo Fisher Scientific). MGB probes are a trademark of Elitech group. The Kits have been internally tested by our quality control. Any responsibility is waived if the warranty of quality control does not refer to the specific Kits. The user is personally responsible for data that he/she will obtain and/or he/she will supply to third parties using these kits. Once the sealed package is opened the user accepts all the conditions without fail; if the package is still sealed the kit can be returned and the user can be refunded. Kits components are intended, developed, designed, and sold for Research Purpose Only. Product claims are subject to change. Therefore, please refer to our website ([www.hyris.net](http://www.hyris.net)) for the most up-to-date information on Hyris Ltd products.

Possible causes	Corrective actions
The <b>Positive Control</b> 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 <b>Negative Control</b> or the <b>qPCR Master Mix</b> 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> .

Document revision Feb 06th, 2020