

bKIT *Silybum marianum*

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

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

HYRIS Asia Pac
Block 998 Toa Payoh North #06-06
Toa Payoh North Industrial Estate
Singapore 318993, Singapore
Phone: +65.8160.7207
Mail: office@hyris.net

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

HYRIS Asia Pac

Block 998 Toa Payoh North #06-06
 Toa Payoh North Industrial Estate
 Singapore 318993, Singapore
 Phone: +65.8160.7207
 Mail: office@hyris.net

www.hyris.net

WARNING AND PRECAUTIONS

Do not mix the components of kits with different batch number. 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. 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 is not responsible for the data resulting from the use of the Kits, for the utilization that the user independently decides to make of them or for the direct or indirect damages possibly resulting from the disclosure or transmission of the data themselves to third parties under any form or circumstance. This clause is automatically accepted by the user when purchasing the Kits.

The patent for performing PCR and Real-Time PCR is held by Hoffmann-La Roche. Authorization to use PCR and Real-Time PCR can be obtained on licence from Hoffmann-La Roche. The product, equipment and information included in the Kits consist of assembled reagents. The licence and authorisation for PCR and Real-Time PCR use are not included in the Kits. The user is responsible for setting prefixed goals, choosing whether or not to perform the PCR or Real-Time PCR reaction and to apply for register his/her own licence. The patent for performing LAMP is held by Eiken, from which authorization to use LAMP can be obtained. The licence for LAMP is not included in the Kits.

The Kits are designed for the services supply. Diagnostic applications that are not research use only require a specific licence for PCR and Real-Time PCR or for LAMP use. The Kits may require the use of Taq Polymerase enzyme and/or fluorochromes, often registered as trademark by companies. TaqMan® is a trademark of Roche Molecular Systems, Inc. FAM, VIC and ROX are a trademark of Applied Biosystems Corporation or its affiliate. Cy5 is a trademark of Amersham Biosciences Ltd. 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.

The Kits are intended for Research Use Only.

***Silybum marianum* L. Gaertn.**

Silybum marianum L. Gaertn. (hereinafter *S. marianum*), or milk thistle, is a plant belonging to the Asteraceae family. It has been known since ancient times for the several beneficial properties exerted by its extracts. The active component is represented by the silymarin flavonoid complex, that has been used for the treatment of liver diseases, diabetic nephropathy and type 2 diabetes mellitus and for anti-inflammatory and anticancer properties ^(1,2,3).

⁽¹⁾ Féher J, Lengyel G. Silymarin in the prevention and treatment of liver diseases and primary liver cancer. *Curr Pharm Biotechnol.* 2012 Jan;13(1):210-7. Review. PubMed PMID: 21466434.

⁽²⁾ Voroneanu, L., Nistor, I., Dumea, R., Apetrii, M., & Covic, A. (2016). Silymarin in Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Journal of Diabetes Research*, 2016, 5147468. <http://doi.org/10.1155/2016/5147468>.

⁽³⁾ O.P. Gupta, S. Sing, S. Bani, N. Sharma, S. Malhotra, B.D. Gupta, S.K. Banerjee, S.S. Handa, Anti-inflammatory and anti-arthritic activities of silymarin acting through inhibition of 5-lipoxygenase, *Phytomedicine*, Volume 7, Issue 1, 2000, Pages 21-24, ISSN 0944-7113, [https://doi.org/10.1016/S0944-7113\(00\)80017-3](https://doi.org/10.1016/S0944-7113(00)80017-3).

Principle

Hydrolysis probe Real-Time PCR assay for the detection of *S. marianum*. The product is intended for research purpose only.

NHPRA validation

In the validation trials performed by NHPRA (Natural Health Product Research Alliance) the following species were tested: *Achillea millefolium*, *Camellia sinensis*, *Cirsium arvense*, *Cirsium* spp., *Curcuma longa*, *Helianthus giganteus*, *Panax ginseng*, *Solidago uliginosa*, *Taraxacum officinale*, *Vitis vinifera*.

bKIT *Silybum marianum* packaging

Part Number: bKTB-SM.02-50

Rt-PCR Master Mix (1 tube)	50 tests
Positive Control (1 tube)	10 tests
Negative Control (1 tube)	10 tests

Part Number: bKTB-SM.02-100

Rt-PCR 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.

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 FAM
- bCUBE® sample loading cartridge or, if using other Real-Time PCR instrument, samples loading support according to the instrument specifications.

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

HYRIS Asia Pac

Block 998 Toa Payoh North #06-06
 Toa Payoh North Industrial Estate
 Singapore 318993, Singapore
 Phone: +65.8160.7207
 Mail: office@hyris.net

www.hyris.net

WARNING AND PRECAUTIONS

Do not mix the components of kits with different batch number. 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. 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 is not responsible for the data resulting from the use of the Kits, for the utilization that the user independently decides to make of them or for the direct or indirect damages possibly resulting from the disclosure or transmission of the data themselves to third parties under any form or circumstance. This clause is automatically accepted by the user when purchasing the Kits.

The patent for performing PCR and Real-Time PCR is held by Hoffmann-La Roche. Authorization to use PCR and Real-Time PCR can be obtained on licence from Hoffmann-La Roche. The product, equipment and information included in the Kits consist of assembled reagents. The licence and authorisation for PCR and Real-Time PCR use are not included in the Kits. The user is responsible for setting prefixed goals, choosing whether or not to perform the PCR or Real-Time PCR reaction and to apply for register his/her own licence. The patent for performing LAMP is held by Eiken, from which authorization to use LAMP can be obtained. The licence for LAMP is not included in the Kits.

The Kits are designed for the services supply. Diagnostic applications that are not research use only require a specific licence for PCR and Real-Time PCR or for LAMP use. The Kits may require the use of Taq Polymerase enzyme and/or fluorochromes, often registered as trademark by companies. TaqMan® is a trademark of Roche Molecular Systems, Inc. FAM, VIC and ROX are a trademark of Applied Biosystems Corporation or its affiliate. Cy5 is a trademark of Amersham Biosciences Ltd. 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.

The Kits are intended for Research Use Only.

(*) 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

Extract DNA from samples according to your usual protocol. If necessary, Hyris can recommend an extraction method. At this purpose, contact us at support@hyris.net.

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 or Positive Control or Negative Control	5 µl
Rt-PCR Mastermix	15 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 *S. marianum*.

Negative Control for *S. marianum*.

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.

Method set-up

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

1. On the bCUBE®

- Login on the bAPP.
- Set-up “New Analysis” and Select the “*Silybum marianum* 2.x” 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 *S. marianum* **Positive Control**.
 “NegCtrl” for the well loaded with *S. marianum* **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.

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

HYRIS Asia Pac
 Block 998 Toa Payoh North #06-06
 Toa Payoh North Industrial Estate
 Singapore 318993, Singapore
 Phone: +65.8160.7207
 Mail: office@hyris.net

www.hyris.net

WARNING AND PRECAUTIONS

Do not mix the components of kits with different batch number. 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. 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 is not responsible for the data resulting from the use of the Kits, for the utilization that the user independently decides to make of them or for the direct or indirect damages possibly resulting from the disclosure or transmission of the data themselves to third parties under any form or circumstance. This clause is automatically accepted by the user when purchasing the Kits.

The patent for performing PCR and Real-Time PCR is held by Hoffmann-La Roche. Authorization to use PCR and Real-Time PCR can be obtained on licence from Hoffmann-La Roche. The product, equipment and information included in the Kits consist of assembled reagents. The licence and authorisation for PCR and Real-Time PCR use are not included in the Kits. The user is responsible for setting prefixed goals, choosing whether or not to perform the PCR or Real-Time PCR reaction and to apply for register his/her own licence. The patent for performing LAMP is held by Eiken, from which authorization to use LAMP can be obtained. The license for LAMP is not included in the Kits.

The Kits are designed for the services supply. Diagnostic applications that are not research use only require a specific licence for PCR and Real-Time PCR or for LAMP use. The Kits may require the use of Taq Polymerase enzyme and/or fluorochromes, often registered as trademark by companies. TaqMan® is a trademark of Roche Molecular Systems, Inc. FAM, VIC and ROX are a trademark of Applied Biosystems or its affiliate. Cy5 is a trademark of Amersham Biosciences Ltd. 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.

The Kits are intended for Research Use Only.

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 *S. marianum* in the **Positive Control** or in the sample under analysis will generate an amplification curve (**Fig. 2**).

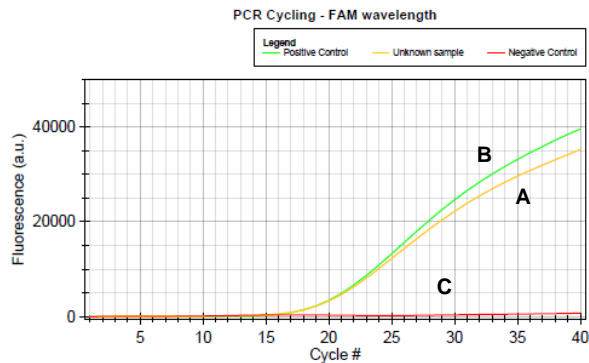


Fig.2. Amplification plot

In the plots, the amplification curve of a *S. marianum* 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	
Positive Control (PosCtrl)	OK	KO
Label meaning	Specific amplification curve present	Specific amplification curve absent

Well type	Possible labels	
Negative Control (NegCtrl)	OK	KO
Label meaning	Specific amplification curve absent	Specific amplification curve present

Well type	Possible labels		
Sample	Present	Absent	Indeterminate
Label meaning	<i>S. marianum</i> is present in the sample	<i>S. marianum</i> is absent from the sample	The test is not conclusive and should be repeated (**)

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

Results for target <i>Silybum marianum</i>	
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.

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

HYRIS Asia Pac

Block 998 Toa Payoh North #06-06
 Toa Payoh North Industrial Estate
 Singapore 318993, Singapore
 Phone: +65.8160.7207
 Mail: office@hyris.net

www.hyris.net

WARNING AND PRECAUTIONS

Do not mix the components of kits with different batch number. 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. 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 is not responsible for the data resulting from the use of the Kits, for the utilization that the user independently decides to make of them or for the direct or indirect damages possibly resulting from the disclosure or transmission of the data themselves to third parties under any form or circumstance. This clause is automatically accepted by the user when purchasing the Kits.

The patent for performing PCR and Real-Time PCR is held by Hoffmann-La Roche. Authorization to use PCR and Real-Time PCR can be obtained on licence from Hoffmann-La Roche. The product, equipment and information included in the Kits consist of assembled reagents. The licence and authorisation for PCR and Real-Time PCR use are not included in the Kits. The user is responsible for setting prefixed goals, choosing whether or not to perform the PCR or Real-Time PCR reaction and to apply for register his/her own licence. The patent for performing LAMP is held by Eiken, from which authorization to use LAMP can be obtained. The license for LAMP is not included in the Kits.

The Kits are designed for the services supply. Diagnostic applications that are not research use only require a specific licence for PCR and Real-Time PCR or for LAMP use. The Kits may require the use of Taq Polymerase enzyme and/or fluorochromes, often registered as trademark by companies. TaqMan® is a trademark of Roche Molecular Systems, Inc. FAM, VIC and ROX are a trademark of Applied Biosystems Corporation or its affiliate. Cy5 is a trademark of Amersham Biosciences Ltd. 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.

The Kits are intended for Research Use Only.

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.

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.

3. An amplification curve 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.

Document revision – February 28th 2019