



NHP
RESEARCH
ALLIANCE

bKIT *Silybum marianum*

Real-Time PCR assay

Code: bKTB-SM.02

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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 (qPCR) assay for the detection of *Silybum 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

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

Part Number: bKTB-SM.02 -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.

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.

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(*) 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

Extract DNA from samples according to your usual protocol. If necessary, Hyris Ltd 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
qPCR 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 *Silybum marianum*.
Negative Control for *Silybum 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 *Silybum marianum* **Positive Control**.
“NegCtrl” for the well loaded with *Silybum marianum* **Negative Control**.
“Sample” for the wells loaded with samples under analysis.

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

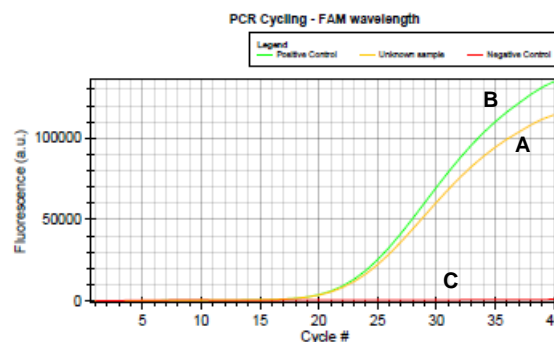


Fig.2. Amplification plot

In the plots, the amplification curve of a *Silybum marianum* containing **sample (A)**, the **Positive Control (B)**, and the **Negative Control (C)** are shown.

- 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	Specific amplification curve present
	KO	Specific amplification curve absent
Negative Control (NegCtrl)	OK	Specific amplification curve absent
	KO	Specific amplification curve present
Sample	Present	<i>Silybum marianum</i> is present in the sample
	Absent	<i>Silybum marianum</i> 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.

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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.

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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