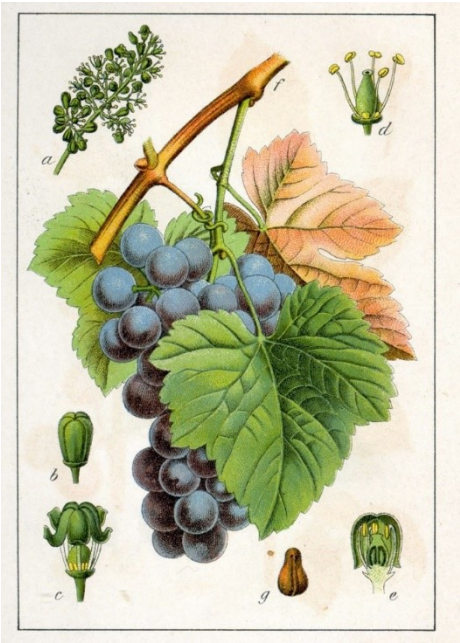
bKIT *Vitis vinifera*

Real-Time PCR assay

Code: bKTB-VV.02



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

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Vitis vinifera

Vitis vinifera is one of the most economical important plant species, since it has been extensively used since ancient times for fruits, juice and wine production. V*itis vinifera* fruit (grape) is also known to contains many bioactive compounds, which have been intensively studied for their antioxidant, anticancer, antibacterial and antidiabetic activities, as well as cardioprotective, hepatoprotective and neuroprotective effects ([1]).

([1]) Nassiri-Asl M, Hosseinzadeh H. Review of the Pharmacological Effects of Vitis vinifera (Grape) and its Bioactive Constituents: An Update. Phytother Res. 2016 Sep;30(9):1392-403. doi: 10.1002/ptr.5644. Epub 2016 May 16. Review. PubMed PMID:27196869.

Principle

Hydrolysis probe Real-Time PCR (qPCR) assay for the detection of *Vitis vinifera*. 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: *Acer negundo, Ampelopsis brevipedunculata, Curcuma longa, Echinocystis lobate*, *Panax quinquefolius, Parthenocissus quinquefolia, Silybum marianum, Vitis berlandieri* x *Vitis riparia* and *Vitis riparia*.

bKIT *Vitis vinifera* packaging

Part Number: bKTB-VV.02-50	
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: bKTB-VV.02-100	
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

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.

(*) 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

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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

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 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 *Vitis vinifera.*
Negative Control for *Vitis vinifera.*
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 "Vitis vinifera 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 *Vitis vinifera.* Positive Control.
"NegCtrl" for the well loaded with *Vitis vinifera.* 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®

a. The presence of the target *Vitis vinifera* in the **Positive Control** or in the **sample** under analysis will generate an amplification curve **(Fig. 2)**

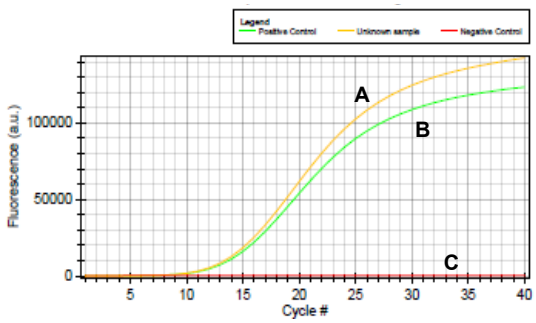

Fig.2. Amplification plot
In the plots, the amplification curve of a *Vitis vinifera* containing **sample** (**A**), the **Positive Control** (**B**), and the **Negative Control** (**C**) are shown.

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**	Specific amplification curve present
	KO	Specific amplification curve absent

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

Well type	Possible labels	Label meaning
Sample	**Present**	*Vitis vinifera* is present in the sample
	Absent	*Vitis vinifera* 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 *Vitis vinifera*		
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

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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 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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