HYRIS
GLOBAL DIAGNOSTICS

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bKIT *Vitis* spp.

Real-Time PCR assay

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Vitis spp.

The genus *Vitis* includes about 60 species ([1]). Among these, *Vitis vinifera* is one of the most economical important species, since it has always been used for fruits, juice and wine production. Other species, such as *Vitis berlandieri* and *Vitis riparia* are usually adopted as rootstock, being more resistant to pests, diseases, and abiotic stress ([2]). In *Vitis* spp. many bioactive compounds have been identified showing antioxidant, anticancer, antibacterial and antidiabetic activities as well as cardioprotective, hepatoprotective and neuroprotective effects ([3,4]).

([1]) Wan Y, Schwaninger HR, Baldo AM, Labate JA, Zhong GY, Simon CJ. A phylogenetic analysis of the grape genus (Vitis L.) reveals broad reticulation and concurrent diversification during neogene and quaternary climate change. BMC Evol Biol. 2013 Jul 5;13:141. doi: 10.1186/1471-2148-13-141. PubMed PMID: 23826735; PubMedCentral PMCID: PMC3750556.
([2]) This P, Lacombe T, Thomas MR. Historical origins and genetic diversity of wine grapes. Trends Genet. 2006 Sep;22(9):511-9. Epub 2006 Jul 26. Review. PubMed PMID: 16872714.
([3]) 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.
([4]) Lu JN, Panchanathan R, Lee WS, Kim HJ, Kim DH, Choi YH, Kim GS, Shin SC, Hong SC. "Anthocyanins from the Fruit of Vitis Coignetiae Pulliat Inhibit TNF-Augmented Cancer Proliferation, Migration, and Invasion in A549 Cells". Asian Pac J Cancer Prev. 2017 Nov 26;18(11):2919-2923. PubMed PMID: 29172259; PubMed Central PMCID: PMC5773771.

Principle

SYBR® Green Real-Time PCR assay for the detection of *Vitis* spp.. The product is intended for research purpose only.

bKIT *Vitis* spp. packaging

Part Number: bK.1/VS-50

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

Part Number: bK.1/VS-100

Rt-PCR Master Mix (2 tubes)	2 x 50 tests
Positive Control (1 tube)	20 tests
Negative Control (1 tube)	20 tests

Specificity

This kit allows the authentication of the genus *Vitis* through a specific SYBR® Green Real-Time PCR assay targeting the trnL gene, which is described as an useful tool for DNA based identification of herbal products ([5]).

([5]) Mishra P, Kumar A, Nagireddy A, Mani DN, Shukla AK, Tiwari R, Sundaresan V. "DNA barcoding: an efficient tool to overcome authentication challenges in the herbal market". Plant Biotechnol J. 2016 Jan;14(1):8-21. doi: 10.1111/pbi.12419. Epub 2015 Jun 16. Review. PubMed PMID: 26079154.

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

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- 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

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

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	3 µl
Rt-PCR Mastermix	17 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* spp..
Negative Control for *Vitis* spp..
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 either on the bPANEL or on the bAPP.
b. Set-up "New Analysis" and Select the "Vitis spp. 1.1" 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* spp. Positive Control.
"NegCtrl" for the well loaded with *Vitis* spp. 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. <u>On a compatible Real-Time PCR instrument</u>
Please, contact us for the protocol set-up on the instrument.

Reading the results

1. <u>On the bCUBE®</u>

a. The presence of the target *Vitis* spp. 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)**.

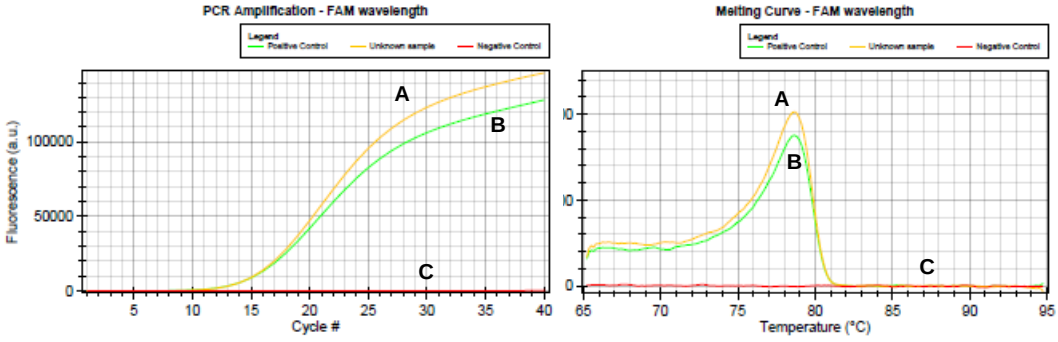

Fig.2. Amplification and melting plots
In the plots, the amplification curve (**Fig. 2a**) and the specific melting peak (**Fig. 2b**) of a *Vitis* spp. containing sample (A), a Positive Control (B), and the Negative Control (C) are shown.

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	**OK**	**KO**
Label meaning	Amplification curve and specific melting peak present	Amplification curve and or specific melting peak absent

Well type	**Possible labels**	
Negative Control	**OK**	**KO**
Label meaning	Amplification curve and specific melting peak absent	Amplification curve and or specific melting peak present

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

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Results for target *Vitis*		
Positive Control	(PosCtrl)	OK
Unknown sample	(Sample)	Present
Negative Control	(NegCtrl)	OK

Fig.3. Analysis results table
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.

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 with a specific melting peak is observed for the Negative Control

Possible causes	Corrective actions
Contamination of the Negative Control or the Rt-PCR 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.