HYRIS
GLOBAL DIAGNOSTICS

TRU-ID

NHP RESEARCH ALLIANCE

bapp.hyris.net/#/bkits

bKIT *Citrus* spp.

PN bKTB-CI.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

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

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

Introduction

The genus *Citrus*, belonging to Rutaceae family, includes many fruit plants such as lemon, lime, mandarin orange, grapefruit, sweet orange. The genus is native to Sub Himalayan tract, China and western Malesia where wild species are indigenous ([1]). Due to their organoleptic properties, their culinary use is distributed all over the world. Moreover, modern researches have demonstrated the presence of many biologically active compounds ([2]) that could act as anticancer, cholesterol-lowering, and antiviral agents.

([1]) Mabberley DJ (1997) A classification for edible Citrus (Rutaceae). Telopea 7: 167–172.
(2) Manners GD. Citrus limonoids: analysis, bioactivity, and biomedical prospects. J Agric Food Chem. 2007 Oct 17;55(21):8285-94. Epub 2007 Sep 25. Review. PubMed PMID: 17892257.

Principle

Hydrolysis probe Real-Time PCR (qPCR) assay for the detection of *Citrus* spp.. 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):1767-1773.
In the validation trials performed by NHPRA (Natural Health Product Research Alliance) the following species were tested: *Citrus x limon, Citrus x aurantiifolia, Citrus x reticulate, Citrus x sinensis, Citrus x paradisi, Atalantia monophylla, Beta vulgaris, Tagetes erecta, Vitis vinifera, Zea mays, Allium sativum, Equisetum hyemale* and *Galium aparine*. The wild *Citrus*-related species *Atalantia monophylla* gave false positive results during the validation trials.

bKIT *Citrus* spp. packaging

Part number: bKTB-CI.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: bKTB-CI.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

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
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

(*) 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

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:

a. Login on the bAPP.
b. Go on bKITs section and select **bKTB-CI.01**.
c. Click the button "Download DNA extraction method".

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 *Citrus* spp..
Negative Control for *Citrus* 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.

Analysis set-up

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

1. On the bCUBE®

a. Login on the bAPP/bPANEL.
   - If you are using the **bAPP**, set-up "New Analysis", flag bKIT, and select "**Botanicals"** → "**bKTB-CI.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 *"***Citrus spp. 1.x***"* (x indicates the number of the last version available) from the "Global recipes" list.

b. Specify the "Well types" for each of the loaded sample as follows (**Fig. 1**):
"PosCtrl" for the well loaded with *Citrus* spp.. Positive Control.
"NegCtrl" for the well loaded with *Citrus* spp.. Negative Control.
"Sample" for the wells loaded with samples under analysis.

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

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

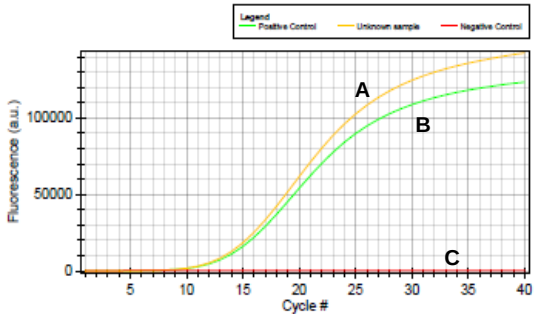

Fig.2. Amplification plot
In the plots, the amplification curve of a *Citrus* spp. containing **sample** (**A**), the **Positive Control** (**B**), and the **Negative Control** (**C**) are shown.

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**	*Citrus* spp. is present in the sample
	Absent	*Citrus* spp. 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.

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

Results for target *Citrus spp.*	
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 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.

Document revision Feb 06th, 2020