bKIT *Camellia* spp.

Real-Time PCR assay

Code: bKTB-CS.02

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Camellia spp.

The genus *Camellia* is the largest genus of the Theacea family. It comprises more than 300 species ([1]). Many of them are appreciated for the ornamental value of their flowers, while *Camellia sinensis* L. is appreciated being the main ingredient of tea, one of the most widespread beverages all over the world. Moreover, some of its components, mainly polyphenolic catechins, are object of investigations for their antioxidant properties and beneficial effects in many diseases associated with free radicals, such as cancer, cardiovascular and neurodegenerative diseases ([2]).

([1]) Mondal TK, Bhattacharya A, Laxmikumaran M, Ahuja PS (2004) Recent advance in tea Biotechnology. Plant Cell Tissue Orga Cult 75:795–856.
([2]) Zaveri NT. Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications. Life Sci. 2006; 78:2073–2080. PMID: 16445946.

Principle

Hydrolysis probe Real-Time PCR (qPCR) assay for the detection of *Camellia* spp.. 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: *Acacia decurrens, Acer negundo, Camellia chrysantha, Camellia japonica, Camellia sinensis, Chenopodium ambrosioides, Curcuma longa, Dendrocalamus strictus, Epilobium angustifolium, Panax ginseng, Silybum marianum, Vaccinium myrtillus* and *Vitis vinifera*.
The validation trials confirmed the ability of the assay to successfully detect *Camellia sinensis* and *Camellia chrysantha;* whereas *Camellia japonica* gave the same behavior of the other non-target species tested.

bKIT *Camellia* spp. packaging

Part Number: bKTB-CS.02 -50	
qPCR Master Mix (1 tube)	50 tests
Positive Control (1 tube)	10 tests
Negative Control (1 tube)	10 tests

Part Number: bKTB-CS.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

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
qPCR 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 *Camellia* spp..
Negative Control for *Camellia* 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 on the bAPP.
b. Set-up "New Analysis" and Select the "Camellia spp. 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 *Camellia* spp. Positive Control.
"NegCtrl" for the well loaded with *Camellia* 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 *Camellia* spp. in the **Positive Control** or in the **sample** under analysis will generate an amplification curve **(Fig. 2)**.

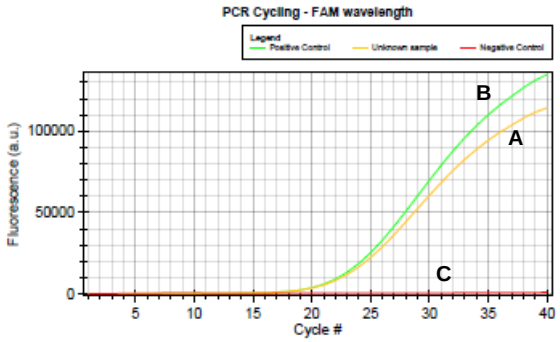

Fig.2. Amplification plot
In the plots, the amplification curve of a *Camellia* spp. containing **sample** (**A**), the **Positive Control** (**B**), and the **Negative Control** (**C**) are shown.

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

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Results for target *Camellia*	
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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