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bKIT *Coffea arabica*

PN bKTB-CA.01

Real-Time PCR assay

Hyris Ltd

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Introduction

Coffea arabica is a plant species of the rubiaceae family. Its cultivation spread from Ethiopia to many tropical countries because of its commercial importance for coffee industry. In fact, due to its beverage quality it is the most representative *Coffea* spp., together with *Coffea canephora* (robusta), used for coffee production.

Principle

Hydrolysis probe Real-Time PCR (qPCR) assay for the detection of *Coffea arabica*. 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: *Coffea arabica*, *Coffea gallieni*, *Coffea excelsa*, *Coffea congensis*, *Coffea liberica*, *Coffea canephora* (robusta), *Actaea racemosa* and *Cynara cardunculus*.

bKIT *Coffea arabica* packaging

Part number: bKTB-CA.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-CA.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.

(*) 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:

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

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 *Coffea arabica*.

Negative Control for *Coffea arabica*.

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.

Analysis set-up

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

1. On the bCUBE®

- Login on the bAPP/bPANEL.
 - If you are using the **bAPP**, set-up "New Analysis", flag bKIT, and select "**Botanicals**" → "**bKTB-CA.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 "**Coffea arabica 1.x**" (x indicates the number of the last version available) 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 *Coffea arabica*. **Positive Control**.
 - "NegCtrl" for the well loaded with *Coffea arabica*. **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 *Coffea arabica* in the **Positive Control** or in the **sample** under analysis will generate an amplification curve (**Fig. 2**)

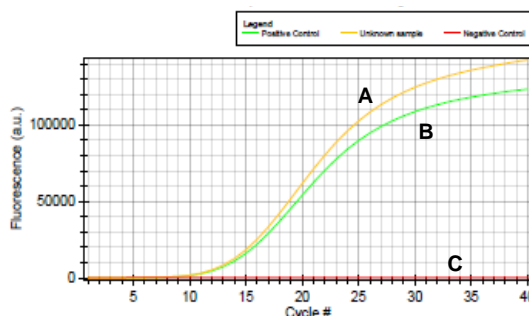


Fig.2. Amplification plot

In the plots, the amplification curve of a *Coffea arabica* 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 |

| 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 | <i>Coffea arabica</i> is present in the sample |
| | Absent | <i>Coffea arabica</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>Coffea arabica</i> | | |
|--|-----------|---------|
| 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 . |

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