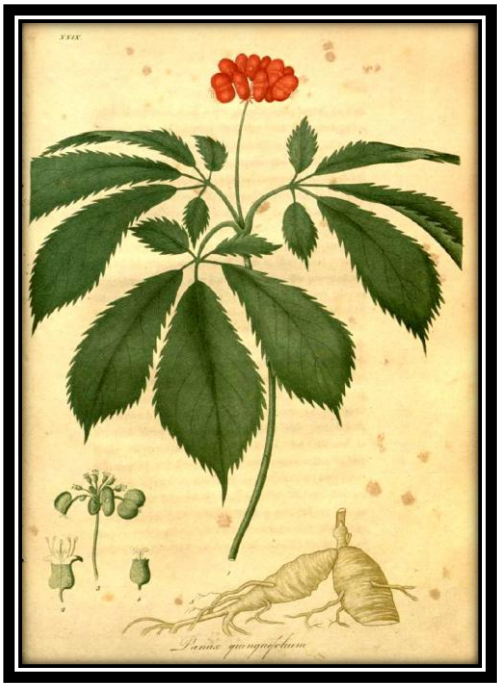
# bKIT *Panax quinquefolius*

## Real-Time PCR assay

**Code: bKTB-PQ.02**

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## *Panax quinquefolius* L.

*Panax quinquefolius* L. (hereinafter *P. quinquefolius*), also called American ginseng, is a perennial plant belonging to the Araliaceae family. Recently, its botanical importance grew, since *P. quinquefolius* has been reported to have several pharmacological effects. Among others, effects on cardiovascular and central nervous systems, and anti-diabetes, anti-tumor, and immunomodulation activities have been described ([1,2]). Its use has increased over the last 100 years and research studies are ongoing to confirm the pharmacological properties of its active components (*).

*(*) The cover image is from ([2]).*

([1]) Punja ZK. American Ginseng: Research Developments, Opportunities, and Challenges. Journal of Ginseng Research. 2011;35(3):368-374. doi:10.5142/jgr.2011.35.3.368.
([2]) Bigelow, Jacob. American Medical Botany: Being a Collection of the Native Medicinal Plants of the United States, Containing Their Botanical History and Chemical Analysis, and Properties and Uses in Medicine, Diet and the Arts, with Coloured Engravings. Vol. 1. Cummings and Hilliard, 1817.

## Principle

SYBR® Green Real-Time PCR (qPCR) assay for the detection of *Panax quinquefolius*. 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: *Ginkgo biloba, Glycine max, Mirabilis jalapa, Panax quinquefolius, Panax trifolius, Phytolacca acinosa, Platycodon grandiflirum* and *Vitis berlandieri x Vitis riparia*.
The validation trials highlighted the needing for further analyses to discriminate between *Panax ginseng* and *Panax quinquefolius.*

## bKIT *Panax quinquefolius* packaging

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

| **Part Number: bKTB-PQ.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 SYBR® Green.
- 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 | 4 µL |
| qPCR Mastermix | 16 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 *Panax quinquefolius.*
Negative Control for *Panax quinquefolius.*
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 "Panax quinquefolius 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 *Panax quinquefolius* Positive Control.
"NegCtrl" for the well loaded with *Panax quinquefolius* 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 *Panax quinquefolius* 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)**.

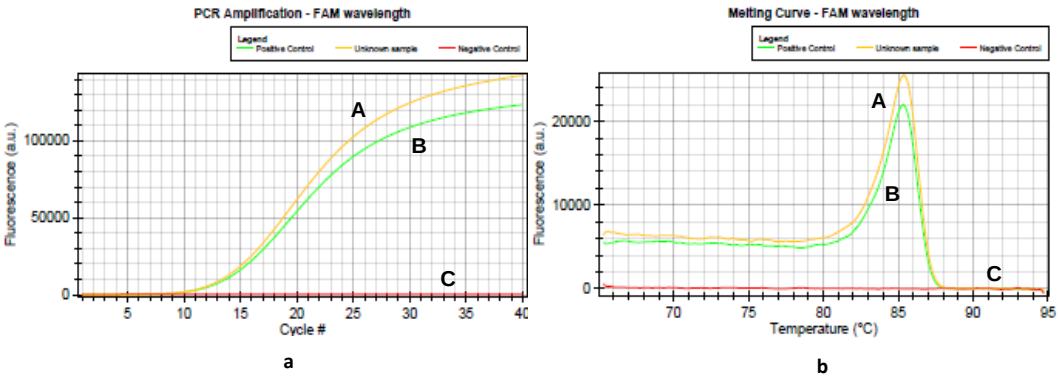


**Fig.2. Amplification and melting plots**
In the plots, the amplification curve (**Fig. 2a**) and the specific melting peak (**Fig. 2b**) of a *Panax quinquefolius* containing **sample** (**A**), the **Positive Control** (**B**), and the **Negative Control** (**C**) are shown.

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** | Amplification curve and specific melting peak present |
| | **KO** | Amplification curve and or specific melting peak absent |

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

| Well type | Possible labels | Label meaning |
|---|---|---|
| **Sample** | **Present** | *Panax quinquefolius* is present in the sample |
| | **Absent** | *Panax quinquefolius* 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](mailto:support@hyris.net).*

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| Results for target *Panax quinquefolius* | |
|---|---|
| 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. |

Document revision May 09 2019.