HYRIS
GLOBAL DIAGNOSTICS

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bKIT *Panax quinquefolius*

Real-Time PCR assay

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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. It was used often in the commercial counterfeiting of *P. ginseng* M.A. Mey., the most wide spread botanical *Panax* sp., due to its similarity at lower price. More recently, its botanical importance grew, since *P. quinquefolius* has been reported to have a number of pharmacological effects analogous and different to those of *P. ginseng* M.A. Mey.. 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 assay for the detection of *P. quinquefolius*. The product is intended for research purpose only.

bKIT *Panax quinquefolius* packaging

Part Number: bK.1/PQ-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/ PQ-100

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

Specificity

The bKIT allows the specific detection of *P. quinquefolius* through the amplification of a specific sequence of a nuclear ribosomal DNA region, which is often used for phylogenetic studies and in identification protocols, since it usually is conserved at intraspecies level ([3,4]).

([3]) Fushimi H, Komatsu K, Isobe M, Namba T. "18S ribosomal RNA gene sequences of three Panax species and the corresponding ginseng drugs." Biol Pharm Bull. 1996 Nov;19(11):1530-2. PubMed PMID: 8951182.
([4]) Komatsu K, Zhu S, Fushimi H, Qui TK, Cai S, Kadota S. "Phylogenetic analysis based on 18S rRNA gene and matK gene sequences of Panax vietnamensis and five related species." Planta Med. 2001 Jul;67(5):461-5. PubMed PMID: 11488463.

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

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- bCUBE® instrument or other Real-Time PCR instrument (**) with filters calibrated 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	4 µl
Rt-PCR 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 *P. quinquefolius*.
Negative Control for *P. 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 either on the bPANEL or on the bAPP.
b. Set-up “New Analysis” and Select the “Panax 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 *P. quinquefolius* Positive Control.
“NegCtrl” for the well loaded with *P. 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

Results analysis

The presence of the target *P. 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 at + 80 °C (± 1.5 °C) **(Fig. 2b)**.

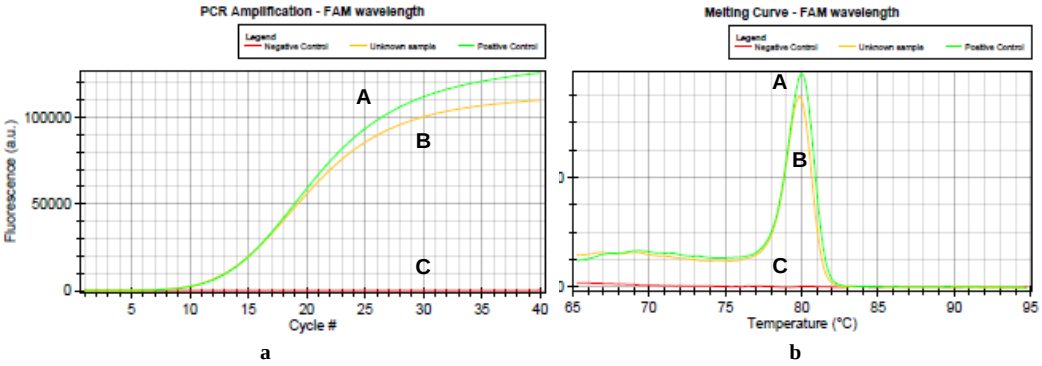

Fig.2. Amplification and melting plots
In the plots, the amplification curve (**Fig. 2a**) and the specific melting peak (**Fig. 2b**) of a *P. quinquefolius* containing sample (A), the Positive Control (B), and the Negative Control (C) are shown.

Troubleshooting

1. <u>Results show no amplification, or anomalous amplification curves</u>

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. <u>No amplification curve is observed for the Positive Control</u>

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. <u>An amplification curve with a specific melting peak is observed for the Negative Control</u>

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.