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# **bKIT *Ginkgo biloba***

LAMP assay

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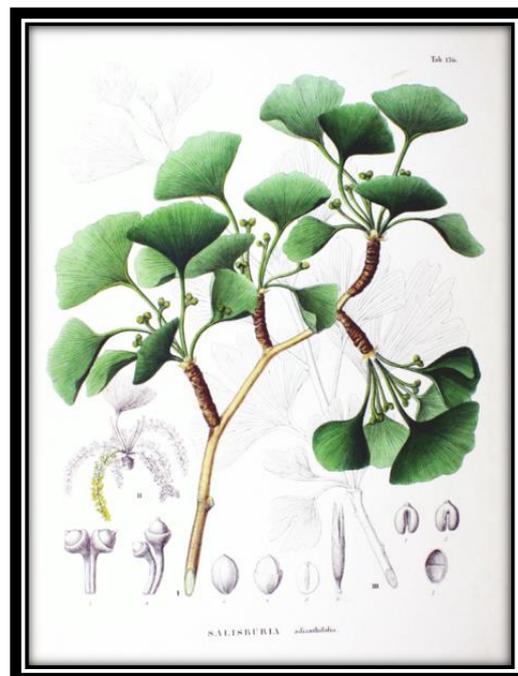
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## Ginkgo biloba

*Ginkgo biloba* (hereinafter *G. biloba*) is universally considered a living fossil, as it has remained essentially unchanged for more than 200 million years <sup>(1)</sup>. *G. biloba* has a long history of use in the Traditional Chinese Medicine (TCM) as treatment for various ailments. Modern researches focused on possible therapeutic effects of *Ginkgo biloba*. Among the most documented, beneficial effects of its extracts have been observed for treatment of chronic schizophrenia and dementia <sup>(2,3)</sup>.

<sup>(1)</sup> Crane PR. , 2013, *Ginkgo* : the tree that time forgot Yale University PressNew Haven

<sup>(2)</sup> Singh, V., Singh, S.P., and Chan, K. 2010. Review and meta-analysis of usage of *Ginkgo* as an adjunct therapy in chronic schizophrenia. *Int. J. Neuropsychopharmacol.* 13: 257–271. doi:10.1017/S1461145709990654. PMID:19775502.

<sup>(3)</sup> Tan MS, Yu JT, Tan CC, Wang HF, Meng XF, Wang C, Jiang T, Zhu XC, Tan L. "Efficacy and adverse effects of *Ginkgo biloba* for cognitive impairment and dementia: a systematic review and meta-analysis." *J Alzheimers Dis.* 2015;43(2):589-603. doi: 10.3233/JAD-140837. Review. PubMed PMID: 25114079.

## Principle

Loop-mediated-isothermal amplification (hereinafter LAMP) assay for the detection of *G. biloba*. The product should be used only for research purposes.

## NHPRA validation

In the validation trials performed by NHPRA (Natural Health Product Research Alliance) the following species were tested: *Cycas revoluta*, *Ephedra aspera*, *Juniperus communis*, *Panax ginseng*, *Panax quinquefolius*, *Pinus strobus*, *Silybum marianum*, *Styphnolobium japonicum*, *Taxus canadensis*, *Vaccinium myrtillus* and *Vitis vinifera*.

## bKIT Ginkgo biloba packaging

### Part Number: bKITB-GB.01-50

LAMP Master Mix (1 tube)	50 tests
Positive Control (1 tube)	50 µl
Negative Control (1 tube)	50 µl

### Part Number: bKITB-GB.01-100

LAMP Master Mix (2 tubes)	2 x 50 tests
Positive Control (1 tube)	100 µl
Negative Control (1 tube)	100 µl

## 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
- Real-time PCR instrument (\*\*\*) with filters calibrated for SYBR®-Green or isothermal amplification instrument

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- Real-time PCR plate or strips according to thermal cycler / isothermal amplification 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

- 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 <b>Positive Control</b> or <b>Negative Control</b>	6 µl
<b>LAMP Mastermix</b>	14 µl
<b>Total Volume</b>	20 µl

## Cartridge set-up

The procedure described is for the bCUBE® cartridge, but, if using a different 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 *Ginkgo biloba*.

**Negative Control** for *Ginkgo biloba*.

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.

## Method set-up

Set up the run method using the following conditions, depending on the instrument you use.

### 1. On the bCUBE®

- Login on the bAPP.
- Set-up “New Analysis” and Select the “*Ginkgo biloba 1.x*” 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 *Ginkgo biloba*. **Positive Control**.  
 “NegCtrl” for the well loaded with *Ginkgo biloba*. **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.

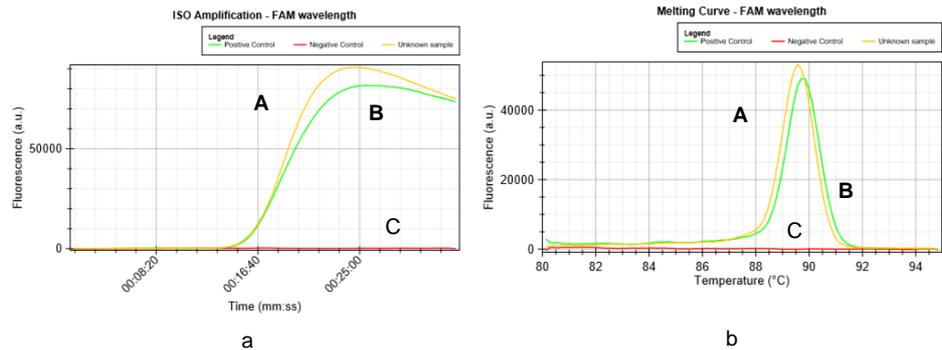
**2. On a compatible instrument**

Please, contact us for the protocol set-up on the instrument.

**Reading the results**

**1. On the bCUBE®**

- a. The presence of the target *Ginkgo biloba* 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 *Ginkgo biloba* containing sample (A), a **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		
Positive Control (PosCtrl)	OK		KO
Label meaning	Amplification curve and specific melting peak present		Amplification curve and/or specific melting peak absent
Well type	Possible labels		
Negative Control (NegCtrl)	OK		KO
Label meaning	Amplification curve and specific melting peak absent		Amplification curve and or specific melting peak present
Well type	Possible labels		
Sample	Present	Absent	Indeterminate
Label meaning	<i>Ginkgo biloba</i> is present in the sample	<i>Ginkgo biloba</i> is absent from the sample	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 <i>Ginkgo biloba</i>		
Positive Control	(PosCtrl)	OK
Negative Control	(NegCtrl)	OK
Unknown sample	(Sample)	Present

Fig.3. Analysis results table

The results table, as reported in the pdf report of the analysis, is shown.

## 2. For the use with a compatible Real-time PCR instrument or isothermal amplification instrument

Please, contact us for the protocol set-up on the instrument and 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 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.

### 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 LAMP 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.

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