bKIT *Zingiber officinale* Rosc.

Real-Time PCR assay

Hyris Ltd

HYRIS Headquarters
Lower Ground Floor, One George Yard,
EC3V 9DF, London, UK
Phone: +44.2036082968
Mail: office@hyris.net

HYRIS Research Center
Corso Garibaldi 60,
Milano 20121, Italy
Phone: +39.02.82951302
Mail: administrator@hyris.net

HYRIS Asia Pac
Block 998 Toa Payoh North #06-06
Toa Payoh North Industrial Estate
Singapore 318993, Singapore
Phone: +65.8160.7207
Mail: office@hyris.net

www.hyris.net

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Lower Ground Floor, One George Yard,
EC3V 9DF, London, UK
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Zingiber officinale Rosc.

Ginger (*Zingiber officinale* Roscoe), a member of the tropical and sub-tropical Zingiberaceae family, has been cultivated for thousands of years for medicinal and culinary purposes. In fact, it is used in Traditional Chinese Medicine (TCM), Ayurvedic and Western herbal medicinal practice to treat headaches, nausea, arthritis, rheumatic disorders and muscular discomfort ([1]). This species contains biologically active constituents, including the main pungent principles: the gingerols and shogaols. These major active components have been found in the fresh rhizome ([2]) and dried ginger ([3; 4]). It has been shown that these components possess various pharmacological and physiological effects including anti-inflammatory, analgesic, antipyretic, gastroprotective, cardiotonic and antihepatotoxic activities ([5]). Due to these properties, in recent years, ginger has gained considerable attention as a botanical dietary supplement in the USA and Europe for its use in the treatment of chronic inflammatory conditions. Despite its widespread medicinal and culinary uses, the authentication of ginger samples remains a difficult issue due to heterogeneity of the plant material and by the purposeful adulteration of some commercial samples.

([1]) Dedov, V.N., Tran, V.H., Duke, C.C., Connor, M., Christie, M.J., Mandadi, S., Roufogalis, B.D., 2002. "Gingerols: a novel class of vanilloid receptor (VR1) agonists." Br. J. Pharmacol. 137 (6), 793–798.
([2]) Govindarajan, V.S., 1982. Ginger-chemistry, technology and quality evaluation: Part I. Crit. Rev. Food Sci. Nutr. 17, 1–96.
([3]) Connell, D.W., Sutherland, M.D., 1969. A re-examination of gingerol, shogaol, and zingerone, the pungent principles of ginger (Zingiber officinale Roscoe). Aust. J. Chem. 22, 1033–1043
([4]) Mustafa, T., Srivastava, K.C., Jensen, K.B., 1993. Drug development report (9): pharmacology of ginger, Zingiber officinale. J. Drug Dev. 6, 25–39.
([5]) Jolad, S.D., Lantz, R.C., Solyom, A.M., Chen, G.J., Bates, R.B., Timmermann, B.N., 2004. Fresh organically grown ginger (Zingiber officinale): composition and effects on LPS-induced PGE2 production. Phytochemistry 65 (13), 1937–1954.

Principle

SYBR® Green Real-Time PCR (qPCR) assay for the detection of *Zingiber officinale*. 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: *Alpinia officinarum, Brassica napus, Camellia sinensis, Curcuma aromatica, Curcuma caesia, Curcuma longa, Curcuma zanthorrhiza, Curcuma zedoaria, Kaempferia galanga, Linum usitatissimum, Panax ginseng, Panax quinquefolius, Serenoa repens, Silybum marianum, Triticum aestivum, Vaccinium myrtillus, Vitis vinifera*.

bKIT *Zingiber officinale Rosc.* packaging

Part Number: bKTB-ZO.01-50

qPCR Master Mix (1 tube)	50 tests
Positive Control (1 tube)	10 tests
Negative Control (1 tube)	10 tests

Part Number: bKTB-ZO.01-100

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

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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.

(*) 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	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 *Zingiber officinale.*
Negative Control for *Zingiber officinale.*
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®

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a. Login on the bAPP.
b. Set-up "New Analysis" and Select the "*Zingiber officinale 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 *Zingiber officinale*. Positive Control.
"NegCtrl" for the well loaded with *Zingiber officinale*. Negative Control.
"Sample" for the wells loaded with samples under analysis.

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 *Zingiber officinale* 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)**.

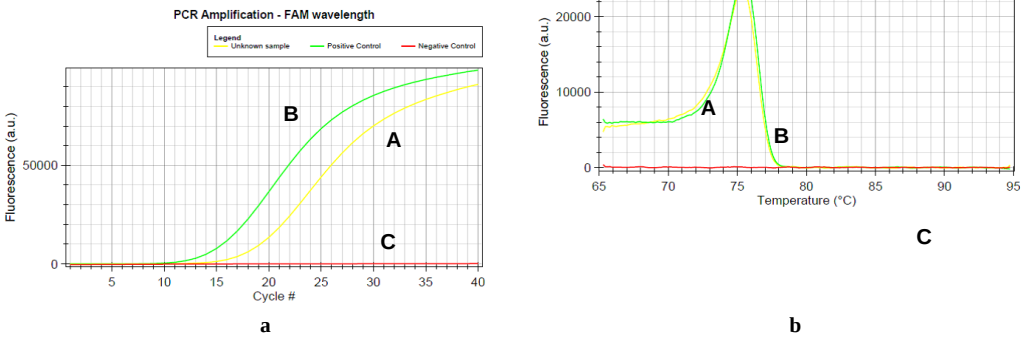

Fig.2. Amplification and melting plots
In the plots, the amplification curve (**Fig. 2a**) and the specific melting peak (**Fig. 2b**) of a *Zingiber officinale* containing sample (A), a Positive Control (B), and the Negative Control (C) are shown.

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	*Zingiber officinale* is	*Zingiber officinale* is	The test is not conclusive

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	present in the sample	absent from the sample	and should be repeated (**)

(**) If the "Indeterminate" classification persists, contact us at support@hyris.net

Results for target *Zingiber officinale*		
Unknown sample	(Sample)	Present
Positive Control	(PosCtrl)	OK
Negative Control	(NegCtrl)	OK

Fig.3. Analysis results table
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.

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 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.

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