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# ***bKIT Zingiber officinale***

**PN bKTB-ZO.01**

Real-Time PCR assay



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## Introduction

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<sup>(1)</sup>.

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<sup>(2)</sup> and dried ginger<sup>(3,4)</sup>. It has been shown that these components possess various pharmacological and physiological effects including anti-inflammatory, analgesic, antipyretic, gastroprotective, cardiotoxic and antihepatotoxic activities<sup>(5)</sup>. 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.

<sup>(1)</sup> 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.

<sup>(2)</sup> Govindarajan, V.S., 1982. Ginger-chemistry, technology and quality evaluation: Part I. Crit. Rev. Food Sci. Nutr. 17, 1–96.

<sup>(3)</sup> 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.

<sup>(4)</sup> Mustafa, T., Srivastava, K.C., Jensen, K.B., 1993. Drug development report (9): pharmacology of ginger, *Zingiber officinale*. J. Drug Dev. 6, 25–39.

<sup>(5)</sup> 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

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](https://doi.org/10.1002/jaoac.11773).

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* packaging

### Part number: bKTB-ZO.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-ZO.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

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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 cyclers.*

**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:

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

**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 <b>Positive Control</b> or <b>Negative Control</b> | 3 µL         |
| qPCR Mastermix   | 17 µL        |
| <b>Total Volume</b>  | <b>20 µL</b> |

**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.

**Analysis set-up**

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

**1. On the bCUBE®**

- a. Login on the bAPP/bPANEL.
  - If you are using the **bAPP**, set-up “New Analysis”, flag bKIT, and select “**Botanicals**” → “**bKTB-ZO.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 “**Zingiber officinale 1.x**” (x indicates the number of the last version available) from the “Global recipes” list.
- b. 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.

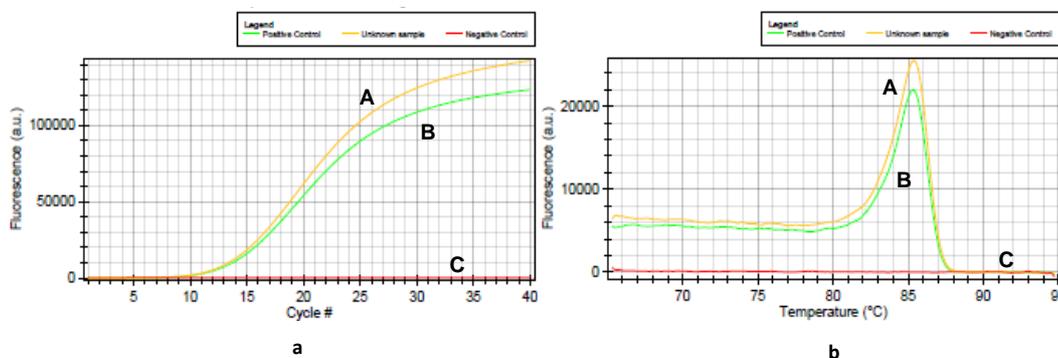
**2. On a compatible Real-Time PCR instrument**

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

**Reading the results**

**1. On the bCUBE®**

- 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), the Positive Control (B), and the Negative Control (C) are shown.

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- 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         | <i>Zingiber officinale</i> is present in the sample    |
|           | Absent          | <i>Zingiber officinale</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](mailto:support@hyris.net).

| Results for target <i>Zingiber officinale</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 <a href="mailto:support@hyris.net">support@hyris.net</a> . |

### 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 <a href="mailto:support@hyris.net">support@hyris.net</a> . |

### 3. An amplification curve with a specific melting peak is observed for the Negative Control

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| Possible causes   | Corrective actions   |
|---|--|
| Contamination of the <b>Negative Control</b> or the <b>qPCR Master Mix</b> 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 <a href="mailto:support@hyris.net">support@hyris.net</a> . |

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