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## **bKIT *Empetrum nigrum***

**PN bKTB-EN.01**

**Real-Time PCR assay**



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## Introduction

*Empetrum nigrum*, also called black crowberry, is a flowering plant of the northern hemisphere belonging to the Ericaceae family. Recently, it gained popularity due to its high content of flavonoids. These compounds are well known for their antioxidant, anti-inflammatory, and anticarcinogenic activities. Moreover, antibacterial and antifungal activities, as well a positive effect on lipid metabolism have been reported for fruits of *Empetrum nigrum* <sup>(1)</sup>.

<sup>(1)</sup> Jurikova T., Mlcek J., Skrovankova S., Balla S., Sochor J., Baron M., Sumczynski D.. "Black Crowberry (*Empetrum nigrum* L.) Flavonoids and Their Health Promoting Activity." *Molecules*. 2016 Dec 7;21(12). pii: E1685. Review. PubMed PMID:27941619; PubMed Central PMCID: PMC6272966.

## Principle

Hydrolysis probe Real-Time PCR (qPCR) assay for the detection of *Empetrum nigrum*. 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](#).

In the validation trials performed by NHPRA (Natural Health Product Research Alliance) the following species were tested: *Calluna vulgaris*, *Camellia sinensis*, *Ceratiola ericoides*, *Cirsium* spp., *Corema conradii*, *Curcuma longa*, *Empetrum atropurpureum*, *Ginkgo biloba*, *Kalmia angustifolia*, *Panax ginseng*, *Panax quinquefolius*, *Serenoa repens*, *Silybum marianum*, *Valeriana officinalis*, *Zingiber officinale*.

The validation trials highlighted the needing for further analyses to discriminate *Empetrum atropurpureum* and *Calluna vulgaris*.

## bKIT *Empetrum nigrum* packaging

### Part number: bKTB-EN.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-EN.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

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 FAM.
- 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 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:

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

## 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>	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 *Empetrum nigrum*.  
**Negative Control** for *Empetrum nigrum*.  
 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.

## Analysis set-up

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

### 1. On the bCUBE®

- Login on the bAPP/bPANEL.
  - If you are using the **bAPP**, set-up "New Analysis", flag bKIT, and select "**Botanicals**" → "**bKTB-EN.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 "**Empetrum nigrum 1.x**" (x indicates the number of the last version available) 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 *Empetrum nigrum*. **Positive Control**.

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“NegCtrl” for the well loaded with *Empetrum nigrum*. **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.

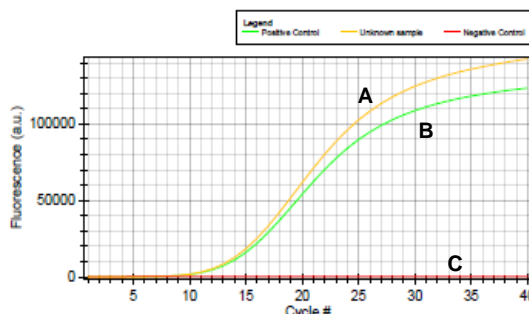
## 2. On a compatible Real-Time PCR instrument

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

## Reading the results

### 1. On the bCUBE®

- The presence of the target *Empetrum nigrum* in the **Positive Control** or in the **sample** under analysis will generate an amplification curve (**Fig. 2**)



**Fig.2. Amplification plot**

In the plots, the amplification curve of a *Empetrum nigrum* containing **sample (A)**, the **Positive Control (B)**, and the **Negative Control (C)** are shown.

- 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	Specific amplification curve present
	KO	Specific amplification curve absent
Well type	Possible labels	Label meaning
Negative Control (NegCtrl)	OK	Specific amplification curve absent
	KO	Specific amplification curve present

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Well type	Possible labels	Label meaning
Sample	Present	<i>Empetrum nigrum</i> is present in the sample
	Absent	<i>Empetrum nigrum</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>Empetrum nigrum</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 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 <a href="mailto:support@hyris.net">support@hyris.net</a> .

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