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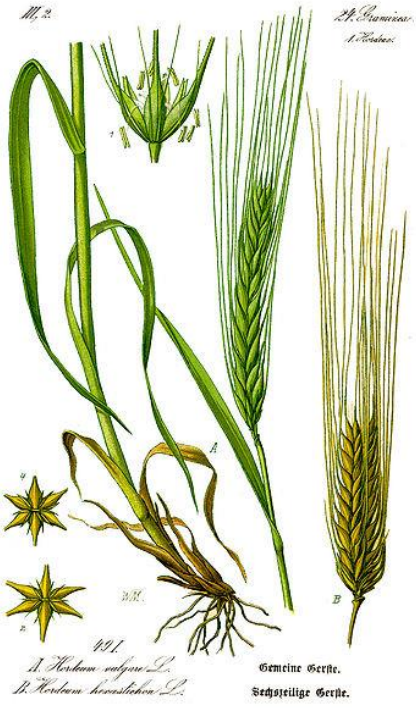
bKIT *Hordeum vulgare*

PN bKTB-HV.01

Real-Time PCR assay



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Introduction

Hordeum vulgare, commonly known as barley, is a plant species belonging to Poaceae family. It was one of the first domesticated grains in the Fertile Crescent about 10000 years ago and it became an important food source all around the world [(1)]. Nowadays, barley usage evolved from food crop to feed and malting/brewing/distilling crop; however, due to its nutritional value and the presence of bioactive compounds, there is renewed interest for food uses [(2)]. In fact, modern researches have demonstrated the presence of many biologically active compounds such as Vitamin E, B-complex vitamins, minerals, phenolic compounds, and β-glucans [(3)].

(1) Badr A, Müller K, Schäfer-Pregl R, et al. On the origin and domestication history of Barley (Hordeum vulgare). Mol Biol Evol. 2000;17(4):499–510. doi:10.1093/oxfordjournals.molbev.a026330
(2) Byung-KeeBaikSteven E.Ullrich. Barley for food: Characteristics, improvement, and renewed interest. Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164-6420, USA. Received 28 August 2007, Revised 8 February 2008, Accepted 14 February 2008, Available online 29 February 2008. https://doi.org/10.1016/j.jcs.2008.02.002
(3) Barley for Brewing: Characteristic Changes during Malting, Brewing and Applications of its By-Products. Mahesh Gupta Nissreen Abu-Ghannam Eimear Gallaghar. First published: 29 April 2010 https://doi.org/10.1111/j.1541-4337.2010.00112.x

Principle

Hydrolysis probe Real-Time PCR (qPCR) assay for the detection of *Hordeum vulgare*. 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: *Hordeum vulgare, Avena fatua, Morus alba, Hordeum jubatum, Hordeum pusillum, Hordeum arizonicum, Hordeum murinum, Hordeum stebbinsii, Triticum aestivum, Allium sativum, Equisetum hyemale,* and *Eleutherococcus senticosus.* Results obtained indicate that the assay allows the successful identification of *Hordeum vulgare*.

bKIT *Hordeum vulgare* packaging

Part number: bKTB-HV.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-HV.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.

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- 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.

(*) 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

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-HV.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 Positive Control or Negative Control	5 µL
qPCR Mastermix	15 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 *Hordeum vulgare.*
Negative Control for *Hordeum vulgare.*
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

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-HV.01**"; alternatively, scan the QRCode on the front page of the present document, or on the bKIT packaging.

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- If you are using the **bPANEL**, set-up "New Analysis" and select *"***Hordeum vulgare 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 *Hordeum vulgare*. Positive Control.
"NegCtrl" for the well loaded with *Hordeum vulgare*. 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 *Hordeum vulgare* in the **Positive Control** or in the **sample** under analysis will generate an amplification curve **(Fig. 2)**

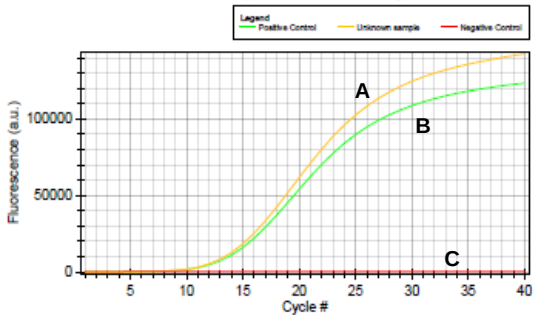

Fig.2. Amplification plot
In the plots, the amplification curve of a *Hordeum vulgare* containing **sample** (**A**), the **Positive Control** (**B**), and the **Negative Control** (**C**) are shown.

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	Label meaning
Positive Control (PosCtrl)	**OK**	Specific amplification curve present
	KO	Specific amplification curve absent

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

Well type	Possible labels	Label meaning
Sample	Present	*Hordeum vulgare* is present in the sample
	Absent	*Hordeum vulgare* 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.

Results for target *Hordeum vulgare*	
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 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 at support@hyris.net.

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 support@hyris.net.

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