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ManKan™ Honey real time PCR kit

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Background

The ManKan™ honey test is a multiplex quantitative polymerase chain reaction (qPCR or real time PCR) test designed for the specific detection, differentiation and quantification of mānuka *(L. scoparium)* DNA from other plant species. Following DNA extraction, it can be used to identify DNA from mānuka pollen in honey and differentiate the similar appearance kānuka (Kan) pollen.

To evaluate the specificity of the "Man" primers and probes, a variety of plant species taxonomically identified were included in the assessment. Over 130 plant specimens representing 36 plant species were tested. From the 70+ plant specimens representing 9 different Leptospermum species, results show the mānuka component of the assay to be highly specific to *L. scoparium* as it does not detect other species such as *L. polygalifolium* (jellybush), *L. laevigatum, L. grandifolium, L. lanigerum* or *L. petersonii.* Leptospermum scoparium samples from a number of different regions in New Zealand were also tested. The assay's analytical specificities was also assessed against other plant species (e.g. Kunzea species, clover, pohutukawa) associated with honey production in New Zealand (~22 different species tested) with no cross reaction observed. Specimens used for specificity testing are archived at the National Forestry Herbarium hosted by Scion Research.

As part of validation and assay application, this assay has been tested on over 800 honey samples from various floral sources and geographic locations. Samples were sourced from two New Zealand flowering seasons, New Zealand industry archives (up to 5 years) and non-New Zealand sourced honeys (e.g. Australia, China, Africa, USA, Europe).

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Method

DNA is extracted from pollen present in honey and then tested using a multiplex qPCR assay (ManKan™ honey test) to detect the levels of mānuka *(Leptospermum scoparium)* DNA. Note: qPCR requires facilities that enable separation of key work flow areas to minimise the likelihood of environmental and cross-contamination.

The protocol has been validated using 10µl reaction volumes on the Eco qPCR system (Illumina) and the Mic cycler (BioMolecular Systems). Other instruments may require verification on the use of such reaction volumes.

Required items:

qPCR

ManKan™ Honey real time PCR kit:

- PCR-grade water
- 20X Oligo Mix
- 5X Mastermix

Kit should be stored long term at -20°C or may be stored at 4°C for up to 6 months from receipt, with no loss of performance.

qPCR plates/tubes as required by qPCR instrument.

qPCR instrument capable of resolving 3 channels (FAM, VIC/HEX and ROX/Texas Red/610)

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DNA extraction

Good quality DNA from small volumes of honey may be extracted using appropriate methods such as the Honey Genomic DNA Mini Kit (dnature).
Please contact dnature for other options when other materials (e.g. plant) are tested.

Reaction mix

Prior to starting, briefly vortex and pulse centrifuge both the primer probe mix and the mastermix.

- Make up a reaction cocktail as follows (adjust volumes for larger reactions)
- Per 10µl reaction (allow extra for pipetting error – 10% additional volume is supplied in the kit):

Water	5.5µl
20X Oligo mix	0.5µl
5X Mastermix	2 µl

Briefly vortex and pulse centrifuge

Dispense 8µl working mastermix per reaction well and 2µl extracted DNA.

Add 2µl ManKan™ positive control DNA to the appropriate positive tube or well and 2µl PCR-grade H2O to the no template control tube or well.

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qPCR protocol

Illumina Eco / BMS Mic

95°C 3 minutes

96°C 6 seconds
62°C 20 seconds
> x40 cycles

(For other instruments, times of 10 seconds (96°C) and 30 seconds (62°C) can be suggested)

Data acquisition at end of 62°C step

Choose the following channels for monitoring:

FAM (manuka – *L. scoparium*)

HEX/VIC (kanuka – *K. ericoides*)

ROX (internal control)

This product has been developed for research purposes only and no diagnostic claims are made.

Analysis

Due to the difference in fluorescent intensities, it can be easier to analyse each channel separately (plots will autoscale)