Bee Pathogen DNA/RNA Extraction Kit

#BDE100 100 preps

Sample: 5-10 bees, processed in buffer. Other insects including wasps and bumblebees may be used also.

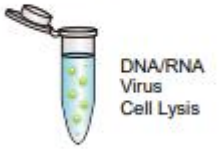
Format: Spin column

Elution volume: 50 µL

Storage: dry at room temperature (15 - 25°C)

Introduction

This kit is designed to purify a range of nucleic acids (including host DNA and RNA) from bees and other insects. It is primarily aimed at extracting a range of pathogens from a single sample, such that the resulting eluate may be used in a range of tests – from RNA viruses to microsporidian nosemas to spore-forming American Foulbrood bacteria.



Contents

DXL Buffer	320 mL
AD Buffer	8 mL (Add 60 mL ethanol)
W1 Buffer	50 mL
Wash Buffer	25 mL (Add 100 mL ethanol)
BD columns (bag)	100
2ml collection tubes	400

Protocol

IMPORTANT BEFORE USE!

• Add absolute ethanol (see the bottle label for volume) to the AD Buffer prior to initial use

• Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use

• Additional requirements: absolute ethanol, microcentrifuge tubes (DNase and RNase-free), 5ml beadbeating tubes (if using beadbeater)

Step 1 Lysis

- Transfer 5 – 10 whole bees to a 5mL beadbeating tube, containing mixture of 0.5mm zirconia silica beads and thin layer 2.3mm zirconia silica beads.[1]
- Add 3 mL of **DXL Lysis Buffer** to the sample then homogenise in beadbeater (typically 3 minutes at maximum speed)
- Incubate at 65°C for >10 minutes, with occasional mixing[2]
- Transfer 1 mL homogenate to a microcentrifuge tube and centrifuge tubes at 12-16,000 x g for 5 minutes.
- Pipette 500 µL supernatant to a new 1.5 mL microcentrifuge tube, discarding pellet.

Step 2 Nucleic Acid Binding

- Add 450 µl of **AD Buffer** (make sure ethanol was added) to the 500 µL lysate.
- Shake / vortex the tube vigorously to mix.
- Place a **VB Column** in a **2 ml Collection Tube**.
- Transfer 600 µl of the mixture (total 950 µL) to the VB Column (do not discard remainder!)
- Centrifuge at 14-16,000 x g for 1 minute.
- Discard the flow-through then place the VB Column back in the 2 ml Collection Tube.
- Transfer the remaining mixture to the VB Column.
- Centrifuge at 14-16,000 x g for 30 seconds.
- Discard the 2 ml Collection Tube containing the flow-through.
- Transfer the VB Column to a new 2 ml Collection Tube.

Step 3 Wash

- Add 400 µl of **W1 Buffer** to the VB Column then centrifuge at 14-16,000 x g for 30 seconds.
- Discard the flow-through then place the VB Column back in a new 2 ml Collection Tube.

- Add 600 µl of **Wash Buffer** (make sure ethanol was added) to the VB Column.
- Centrifuge at 14-16,000 x g for 30 seconds.
- Discard the flow-through and place the VB Column back in the same (or new) 2 ml Collection Tube.
- Add 200 µl of **Wash Buffer** to the VB Column and centrifuge as before for 30 seconds. Discard flow-through and place VB column back in collection tube
- Centrifuge at 14-16,000 x g for 2 minutes to dry the column matrix.

Step 4 DNA/RNA Elution

- Place the dried VB Column in a clean 1.5 ml microcentrifuge tube.
- Add 50 µl of RNase-free Water to the CENTRE of the VB Column matrix[3].
- Let stand for at least 3 minutes to ensure the RNase-free Water is absorbed by the matrix.
- Centrifuge at 14-16,000 x g for 1 minute to elute the purified nucleic acid.

Nucleic acid is ready for PCR

Data

This kit has been tested on a range of bee pathogens including nosemas, Viruses (Black Queen Cell Virus and Deformed Wing Virus used as examples) and American Foulbrood (AFB). The kit has been tested along side our in-house developed laboratory protocol with excellent performance (equivalent sensitivity).

Notes

[1] If not using a beadbeater then mortar and pestle may be used. Pulverise the bees using liquid nitrogen, before adding the 3 mL of DXL buffer and transfer to a 5 mL tube.

[2] Samples may be incubated for up to 1 hour with no detrimental effect.

[3] It can help to form a 'hanging drop' with the 50 µL of elution buffer, before dispensing to centre of VB column matrix. This will help ensure maximum recovery of nucleic acids.