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APPLICATION NOTE No. 380

Purification of Genomic DNA from Honey Bees, Pollen and Honey with the NucleoMag® VET Kit on the ep*Motion*® 5075t/m

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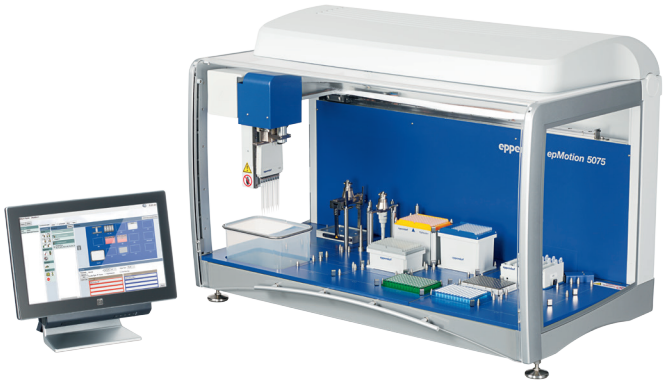
Abstract

The decline of pollinator populations worldwide and their significance in agriculture set the purification of genomic DNA from honey bees, pollen or honey more into focus of research laboratories. The worldwide emerge of honey bee pathogens requires also a simultaneous isolation of host and pathogen nucleic acids. Automation facilitates the purification with an increasing sample number, especially for versatile sample material like pollen, honey or the honey bee itself. The NucleoMag® VET Kit from MACHEREY-NAGEL is here adapted to the ep*Motion* 5075t/m. The combination of the ep*Motion* 5075t/m and the extraction kit allows an efficient walk away purification of DNA in less than 120 minutes.

Introduction

The procedure of the NucleoMag® VET kit is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions.
Whole bees, pollen or honey are prelyzed with PBS buffer, Proteinase and DTT. Subsequently the samples were treated with Lysis buffer VL. The contaminants are removed through two washing steps with wash buffer VEW 1 and VEW 2. Salts are subsequently removed with an additional wash step using 80% ethanol. The purified DNA is eluted and can be used directly as a template for qPCR, next generation sequencing, or any kind of enzymatic reactions.

A purification process with 96 honey bees, pollen or honey samples with re-use tip function for the wash steps requires 240 x 1000 µl tips and 98 x 300 µl tips.
This application note describes the configuration and preparation of the ep*Motion* 5075m/t to automate the NucleoMag® VET kit.



Materials and Methods

Required labware
> Eppendorf ep*Motion* 5075t or 5075m
> Dispensing Tool TM 1000-8
> Dispensing Tool TM 300-8
> Reservoir Rack
> Reservoirs 30 mL / Reservoirs 100 mL
> Reservoir 400 mL
> NucleoMag® Sep (Magnetic separator)
> NucleoMag® VET Kit

Required consumables
> epT.I.P.S® Motion 1000 µL with filter
> epT.I.P.S Motion 300 µL with filter
> Eppendorf Deepwell Plate 96/2000 µL
> Eppendorf Microplate

Samples
> Honey bees
> Pollen
> Honey

Method
This protocol is developed to process up to 96 samples in parallel on the ep*Motion* 5075m or 5075t workstation. This kit is suitable for up to 90 to 100 mg of bees (one bee), 30 to 40 mg pollen or 10 g honey. The samples require different prelysis steps. For honey bees extraction: 90 to 100 mg of the bees were homogenized with 400 µL PBS and 20 µL Proteinase K and 20 µL DTT (Dithiothreitol) with a 3 to 4 mm steal bead for 3 min at maximum speed in a TissueLyser or something similar. This is followed by a shaking step at 56 °C for 2 h. A filtration or centrifugation step clears the prelysate. After the prelysis steps, 220 µL of the clear solution is prefilled into each well of the separation plate. All subsequent steps are automated and will be carried out in this plate. This includes dispensing of buffers and beads, removal of the supernatants as well as transport, temperature incubation and mixing steps.
After the prelysis step, the lysis buffer VL is added. After a mixing and heating step, magnetic beads and binding buffer are added. During a mixing and incubation step, the DNA is bound to the magnetic beads. Beads are separated on a magnetic plate adapter and the supernatant is removed. Unspecific bound contaminants are removed by several washing steps with wash buffers VEW1 and VEW2. Remaining salts are removed with an additional wash step with 80% ethanol. After the last washing step, residual ethanol is removed in a drying step of 10 min at 56 °C. In a last step, the eluate is transferred to a dedicated elution plate.

A purification process with 96 samples with re-use tip function for the wash steps requires 240 x 1000 µl tips and 98 x 300 µl tips and takes 2 h.
For the method, the following positions of the worktable are occupied:

Position	Labware
A2	300 µL filtertips
A3	300 µL filtertips
TMX	Separation Plate (Lysed samples)
B1	1000 µL filtertips
B2	1000 µL filtertips
B3	1000 µL filtertips
C2	Liquid Waste (400 mL reservoir)
C3	NucleoMag® Sep
C4	Reagent reservoirs
C5	Elution Plate

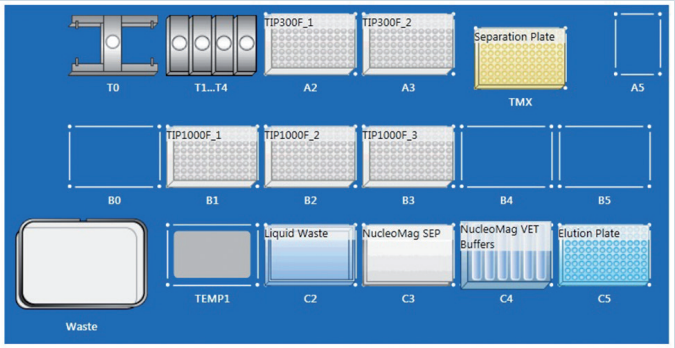


Figure 1: Worktable allocation

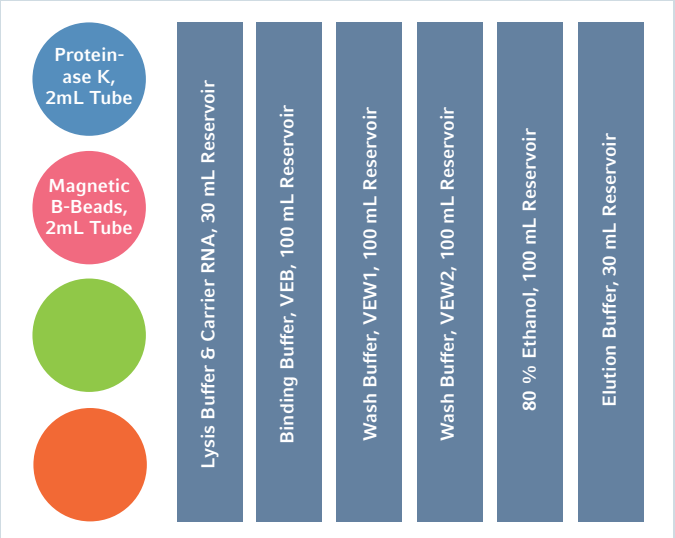


Figure 2: ReservoirRack

Results and Discussion

Purification results from different material, like honey bees, pollen and honey: Genomic DNA acquired with the aforementioned method was analyzed by gel electrophoresis of 10 µL eluate via 1% TAE agarose gel; yield and purity were determined by UV spectroscopy with Synergy™ HT Multi-detection microplate reader (Biotek®). Furthermore a qPCR with SensiFast® Probe Lo-Rox Kit (Bioline) on an Applied Biosystems® 7500 was used to check for the absence of inhibitors.

Yield and the purity of honey bees

Genomic DNA was isolated from honey bees with an average DNA yield of 15,3 µg per sample. Yield and the purity of honey bees is shown in figure 3 and 4. As pathogens are currently great threats for honey bee populations worldwide. the extraction of a viral model RNA (MS2) was performed to demonstrate that the simultaneous extraction of genomic DNA and viral RNA/DNA is possible.

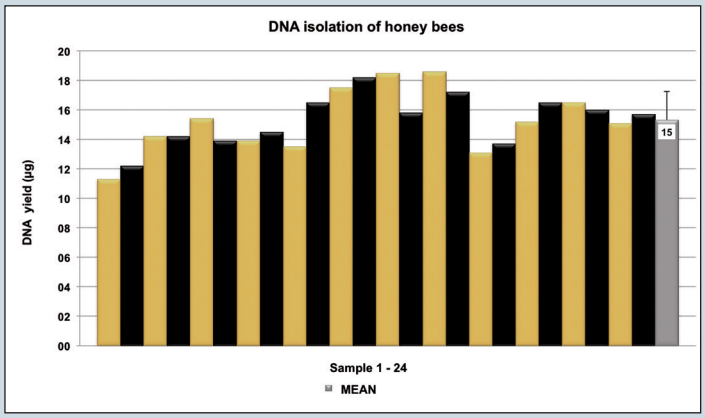


Figure 3: Yield determined by UV spectroscopy

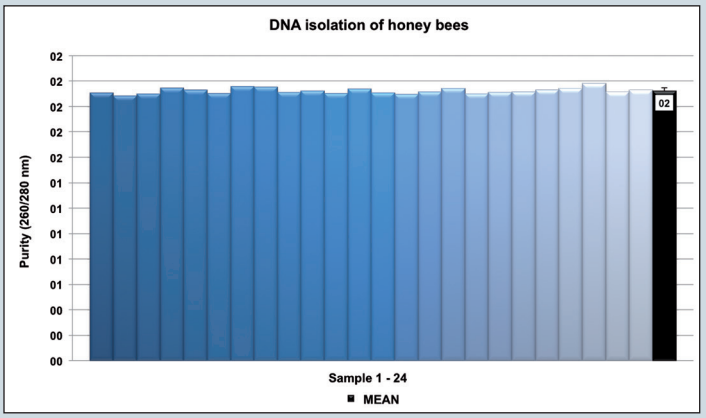


Figure 4: Purity were determined by UV spectroscopy

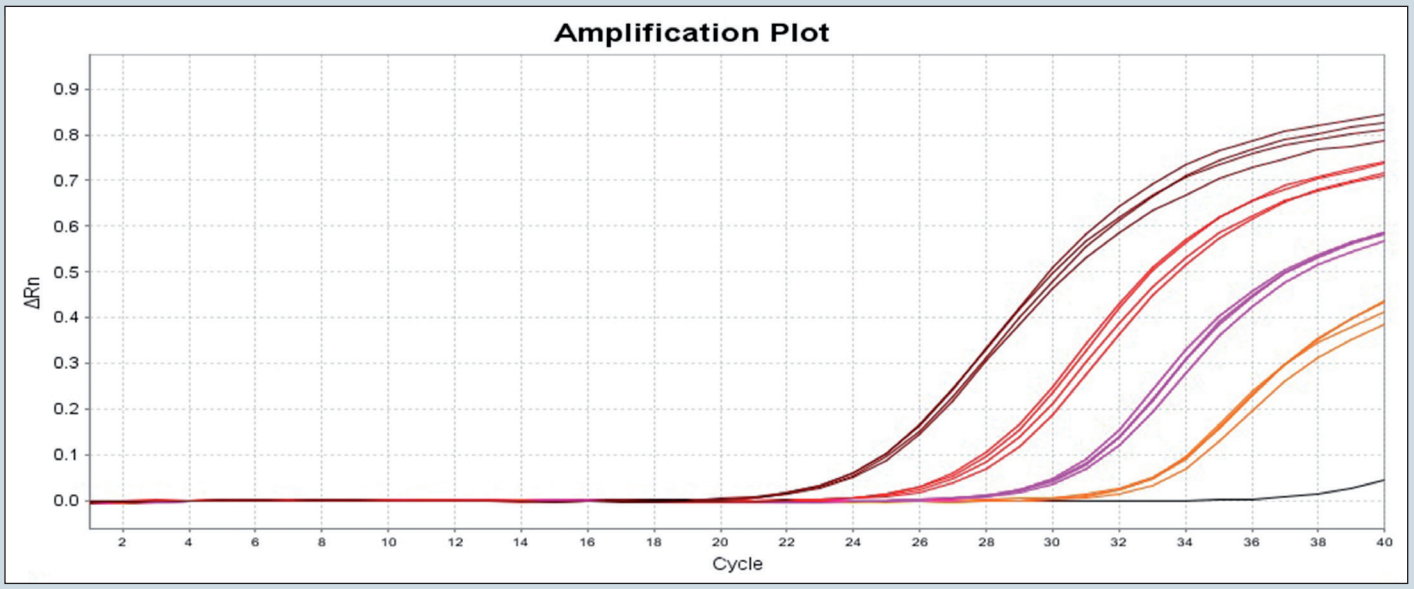


Figure 5: Amplification plot of four different MS2-RNA-dilutions (4000pg, 400pg, 40pg and 4pg) from honey bee samples. MS2 amplicon size is about 200 bp. 4 µL of selected eluates were assayed in a quantitative PCR with a Taqman probe

Yield and purity of pollen

DNA was isolated from pollen with an average yield of 6 µg per sample. Yield and purity is shown in figure 6 and 7. The high standard deviation is caused due to the heterogeneity of the pollen samples. The qPCR in figure 8 results demonstrate that the isolated DNA does not include any PCR-inhibition and is suitable for downstream-analysis (e.g. plant species determination).

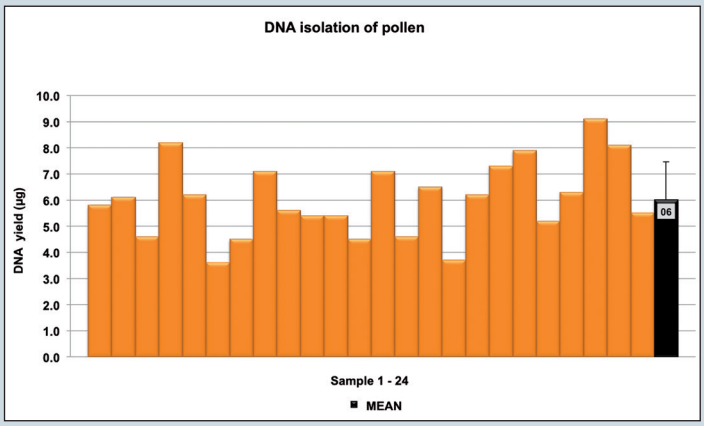


Figure 6: Yield determined by UV spectroscopy

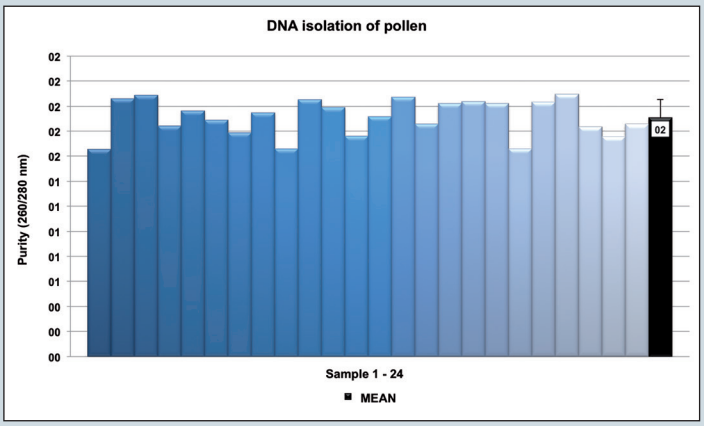


Figure 7: Purity determined by UV spectroscopy

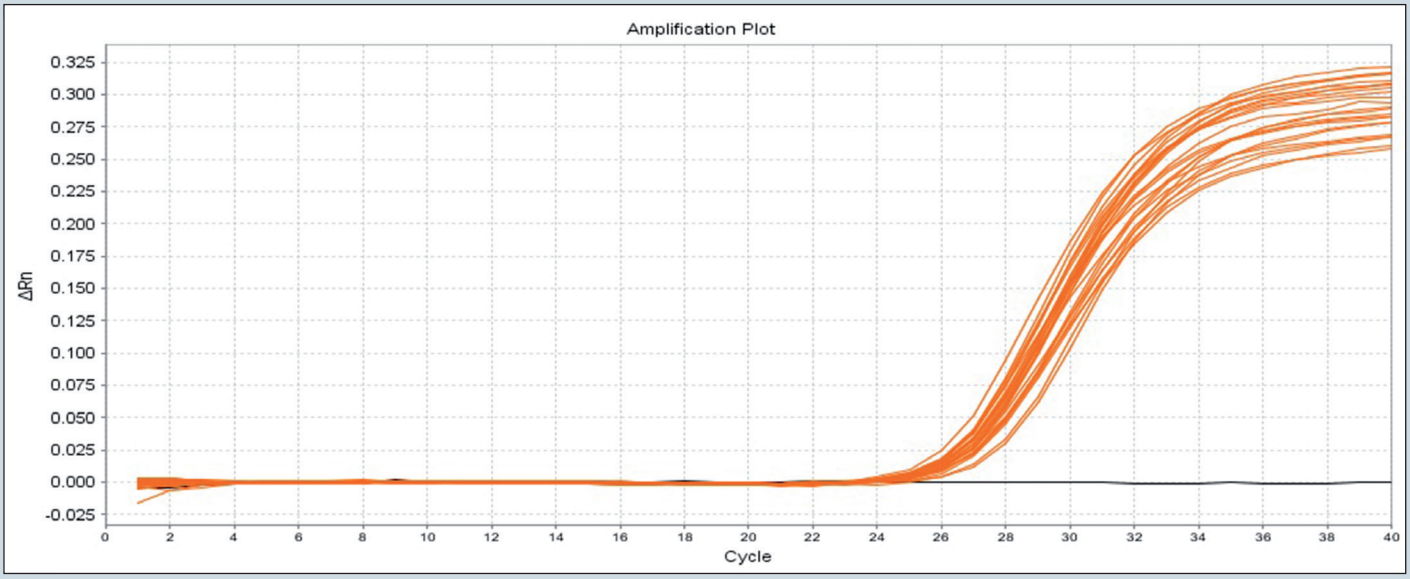


Figure 8: Amplification plot of extracted DNA from pollen samples using a beta actin amplicon size about 100 bp (plant specific). 4 µL of selected eluates were assayed in a quantitative PCR with a Taqman probe.

Yield and purity of honey

Yield and the purity of isolated DNA from honey samples show a successful extraction. The qPCR results demonstrate that the isolated DNA does not include any PCR-inhibitor and is suitable for downstream-analysis.

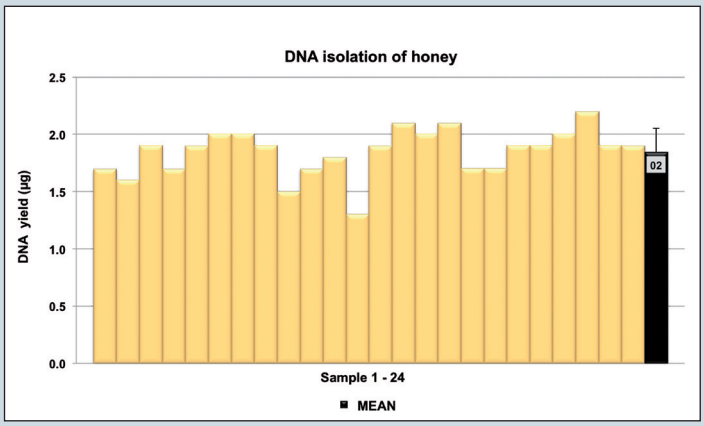


Figure 9: Yield determined by UV spectroscopy

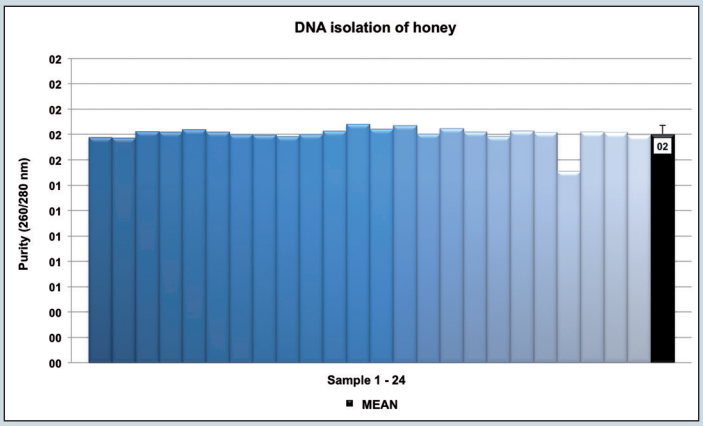


Figure 10: Purity determined by UV spectroscopy

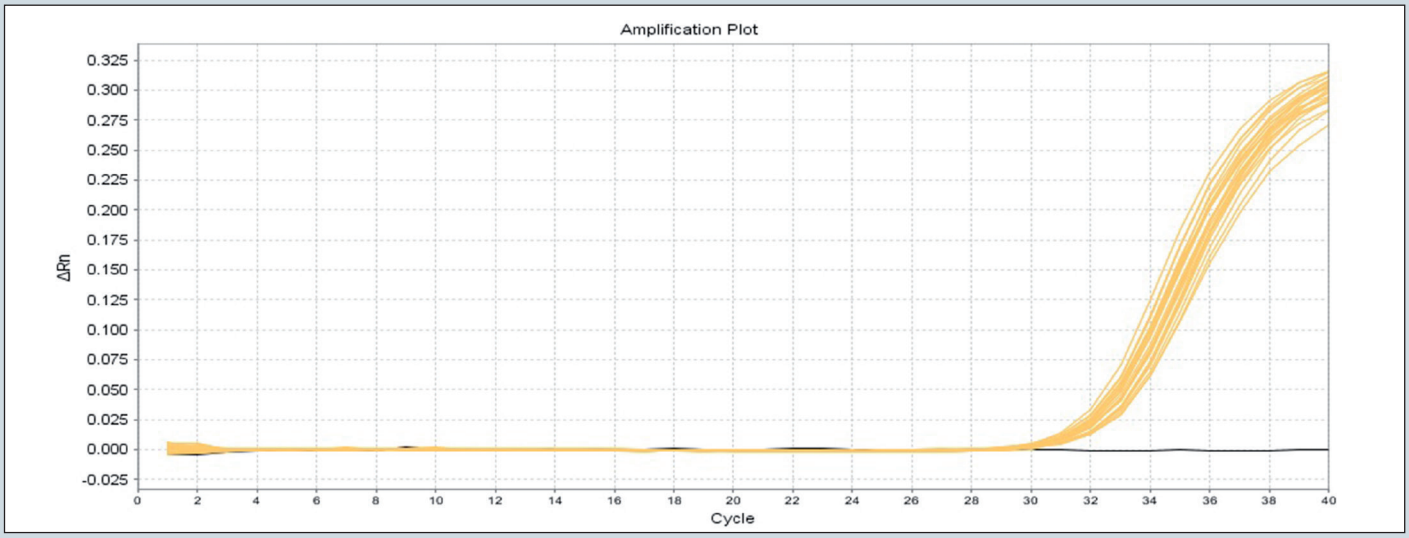


Figure 11: Amplification plot of extracted DNA from honey samples using a beta actin amplicon size about 100 bp (plant specific). 4 µL of selected eluates were assayed in a quantitative PCR with a Taqman probe.

Cross-contamination

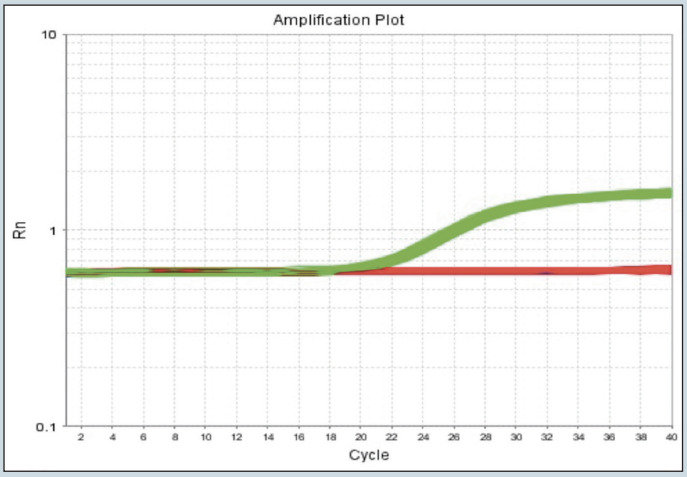


Figure 12: Cross-contamination check using a checkerboard-like pattern and with MS2-RNA as sample material. No PCR amplification could be observed in eluates from negative controls. MS2 amplicon size is about 200 bp. 4 µL of selected eluates were assayed in a quantitative PCR with a Taqman probe.

Conclusion

The above results show that the combination of the NucleoMag® VET kit and the ep*Motion* 5075t/m reliably delivers high yields and of high quality DNA - from different sample material, like honey bees, pollen and honey.
No cross contamination is detectable. The purified DNA is suitable for a full range of downstream methods.

The results from the qPCR, purity and yield as well show the performance of the described procedure. The total time to process 96 samples is 120 minutes.
The use of Eppendorf SafeRack along with the re-use function, have a positiv impact on the cost per sample.

Ordering information

Description	Order no. International
ep*Motion*® 5075t	5075 000.302
ep*Motion*® 5075m	5075 000.305
ReservoirRack	5075 754.002
TM 1000-8 Dispensing tool	5280 000.258
TM 300-8 Dispensing tool	5280 000.231
epT.I.P.S® Motion 1000 µL SafeRack with filter	0030 014.650
epT.I.P.S® Motion 300 µL with filter	0030 014.456
Reservoir 30 mL	0030 126.505
Reservoir 100 mL	0030 126.513
Reservoir 400 mL	5075 751.364
Deepwell Plate 96/2000 µL	0030 501.306

MACHEREY-NAGEL	
NucleoMag® VET	REF 744200.1/4
NucleoMag® SEP	REF 744900

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