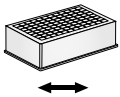
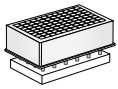
Support protocol for isolation of DNA from insects using the kit NucleoMag® DNA Bacteria (Rev. 02, April 2023)

Protocol at a glance using NucleoSpin® Bead Tube Type D

This protocol is only a supplement to the kit's general user manual. Please refer to the kit manual for more detailed information regarding safety instructions, product-specific disclaimers, and especially preparations needed before starting the procedure. The latest version of the user manual is available at ***www.mn-net.com/usermanuals*** *or can be requested from our technical service (support@mn-net.com). Safety data sheets (SDS) can be downloaded from* ***www.mn-net.com/MSDS****.*

For additional equipment and hardware requirements, refer to section 1.2, 2.4.1, and 2.4.2 of the manual respectively.

For detailed information on each step, see below

1	**Prepare sample**	Place < 40 mg insect material (wet weight) in a NucleoSpin® Bead Tube Type D 140 µL IML	
2	**Lyse sample**	10 µL Liquid Proteinase K 2.5 µL Liquid RNase A Agitate on a swing mill or similar device 0.5–15 min 11,000 x *g*, 30 s	
3	**Adjust binding conditions**	320 µL IML Vortex 3 s, 11.000 x *g*, 30 s	
4	**Bind** DNA to NucleoMag® B-Beads	Up to 500 µL lysate 24 µL NucleoMag® B-Beads 320 µL IMB	
		Mix by shaking for 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 5 min separation	

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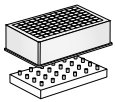
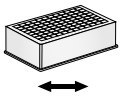
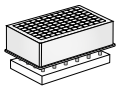
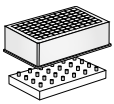
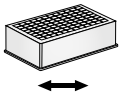
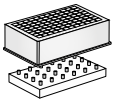
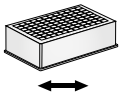
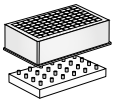
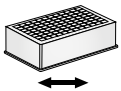
5	**Wash** with IMW	Remove Square-well Block from NucleoMag® SEP 600 µL IMW	
		Resuspend: Shake 2 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
6	**Wash** with 80 % ethanol	Remove Square-well Block from NucleoMag® SEP 600 µL 80 % ethanol	
		Resuresuspend: Shake 2 min at RT *(Optional: Mix by pipettingup and down)*	
		Remove supernatant after 2 min separation	
7	**Wash** with 80 % ethanol	Remove Square-well Block on NucleoMag® SEP 600 µL 80 % ethanol	
		Resuspend: Shake 2 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
8	**Dry** the beads	10 min at RT	
9	**Elute** DNA	Remove Square-well Block from NucleoMag® SEP **50–200 µL IME** *(Optional: Elute at 56 °C)*	

Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	
Separate 2 min and transfer DNA into elution plate	

Detailed protocol using NucleoSpin® Bead Tube Type D

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.5 of the manual). It is recommended to use a Square-well Block for separation (see section 2.5 of the manual). Alternatively, isolation of DNA can be performed in reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

Before starting the preparation:

- Check if Buffer IMW was prepared according to section of the manual.
- Check section 2.4.3 of the manual for additional information regarding time and frequency of disruption for sample material lysis

1 Prepare sample

Place the insect sample to a NucleoSpin® Bead Tube Type D.

Up to approximately 40 mg of wet weight insect sample can be processed. Remove excess liquid (e.g., water, ethanol) from the sample, with a filter paper.

Add **140 µL Buffer IML** to the sample.

2 Lyse sample

Add **10 µL , 2.5 µL Liquid RNase A** and close the tube.

Agitate the NucleoSpin® Bead Tube Type D on the MN Bead Tube Holder in conjunction with Vortex-Genie®, swing mill or similar device for **0.5–15 min** (e.g., 4 min for the MN Bead Tube Holder).

Note: Please refer to section 3 for sample disruption in a 96-well format.

Note: Optimal agitation duration, speed / frequency depends on the device used. For the MN Bead Tube Holder it is approximately 12 min; in a mixer mill MM200, MM300, MM400 (Retsch®), e.g., 40 s at 20 Hertz is suitable (see section 2.4.3 of the manual). On a swing mill, position of the tube in the mill can considerably influence the result. Please refer to the instruction manual of the device used. Respect warnings in section 1.2 and 2.4.3 of the manual if other devices are intended to be used!

Centrifuge the NucleoSpin® Bead Tube D for **30 s** at **11,000 x *g*** to clean the lid.

Attention: Do neither centrifuge at higher g-force, nor for longer than 30 s because this might damage the NucleoSpin® Bead Tubes.

3 Adjust binding conditions

Add **320 µL Buffer IML** and mix (e.g., vortex for 3 s).

Note: Steel beads should be agitated in the tube; some residual pellet (cell debris) may remain on the bottom of the tube.

Centrifuge for **30 s** at **11,000 x** *g*.

Note: This centrifugation step is performed in order to clean the lid and sediment steel beads and cell debris.

Attention: Do neither centrifuge at higher g-force, nor for longer than 30 s because this might damage the NucleoSpin® Bead Tubes.

4 Bind DNA to NucleoMag® B-Beads

Transfer **up to 500 µL lysate to a Square-well Block. Add 24 µL of NucleoMag® B-Beads** and **320 µL of Binding Buffer IMB**. Mix by pipetting up and down 6 times and **shake** for **5 min** at **room temperature**. Alternatively, when processing the kit without a shaker, pipette up and down 10 times and incubate for 5 min at room temperature.

Note: NucleoMag® B-Beads and Buffer MWA2 can be premixed.

Be sure to resuspend the NucleoMag® B-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has formed.

Separate the magnetic beads against the side of the wells by placing the Squarewell Block on the NucleoMag® SEP magnetic separator. Wait at least 5 min until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

Note: Do not disturb the attracted beads while aspirating the supernatant. The magnetic pellet might not be visible in this step. Remove supernatant from the opposite side of the well.

5 Wash with IMW

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL Buffer IMW** to each well and resuspend the beads by shaking until the beads are resuspended completely **(1–3 min)**. Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2–5 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

6 Wash with 80 % ethanol

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL 80 % ethanol** to each well and resuspend the beads by shaking until the beads are resuspended completely **(1–3 min)**. Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

7 Wash with 80 % ethanol

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Add **600 µL 80 % ethanol** to each well and resuspend the beads by shaking until the beads are resuspended completely **(1–3 min)**. Alternatively, resuspend beads completely by repeated pipetting up and down.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnet. Remove and discard supernatant by pipetting.

8 Air dry magnetic beads

Remove the Square-well Block from the NucleoMag® SEP magnetic separator.

Air dry the magnetic beads for **10–15 min** at **room temperature.**

9 Elute DNA

Add desired volume of **Buffer IME (50–200 µL)** to each well of the Square-well Block and resuspend the beads by shaking **5 min** at room temperature. Alternatively, resuspend beads completely by repeated pipetting up and down and incubate for **5–10 min** at **room temperature** or **56 °C**.

Separate the magnetic beads by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **2 min** until all the beads have been attracted to the magnets. Transfer the supernatant containing the purified DNA to suitable elution plate.

Detailed protocol using MN Bead Plate Type D

This protocol is designed for magnetic separators with static pins (e.g., NucleoMag® SEP) and suitable plate shakers (see section 2.5 of the manual). It is recommended using a Square-well Block for separation (see section 2.5 of the manual). Alternatively, isolation of DNA can be performed in reaction tubes with suitable magnetic separators. This protocol is for manual use and serves as a guideline for adapting the kit to robotic instruments.

- For hardware requirements, refer to section 2.4.1 of the manual and 2.4.2 of the manual.

1 Prepare sample

Place individual insect samples into each well of the MN 96 Bead Plate Type D.

Up to approximately 40 mg of wet weight insect sample can be processed. Remove excess liquid (e.g., water, ethanol) from the sample, with a filter paper.

Add **140 µL Buffer IML** to each sample.

2 Lyse sample

Add **10 µL , 2.5 µL Liquid RNase A** and seal the tubes strips tightly with the cap strips.

Agitate the MN 96 Bead Plate Type D on a swing mill or similar device for approx. **2 x 10–60 s** at **20 Hertz**.

Note: Re-orient MN 96 Bead Plates vertically for 180° after the first disruption time. Samples which have been the closest to the machine body should be now the furthest apart.

Note: Optimal agitation duration, speed / frequency depends on the device used. For the mixer mill MM300 (Retsch®), e.g., 2 x 20 s at 20 Hertz is suitable (see section 2.4 of the manual). On a swing mill, position of the tube in the mill can considerably influence the result. Please refer to the instruction manual of the device used. Respect warnings in section 1.2 and 2.4.3 of the manual if other devices are intended to be used!

Centrifuge the MN 96 Bead Plate D for **5 min** at **2,000 x *g*** to clean the lid

Attention: Do neither centrifuge at higher g-force, nor for longer than 30 s because this might damage the MN 96 Bead Plate.

3 Adjust binding conditions

Add **320 µL Buffer IML**, seal the tubes strips with cap strips and **mix** (e.g., vortex for 3 s or invert).

Note: Steel beads should be agitated in the tube; some residual pellet (cell debris) may remain on the bottom of the tube.

Centrifuge for **5 min** at **2,000 x** *g*.

Note: This centrifugation step is performed in order to clean the lid and sediment steel beads and cell debris.

Attention: Do neither centrifuge at higher g-force, nor for longer than 30 s because this might damage the MN 96 Bead Plate.

4 Bind DNA to NucleoMag® B-Beads

Transfer **up to 500 µL lysate to a Square-well Block**. Avoid aspiration of cellular debris.

Note: Individual tubes strips can be removed from the Rack of Tube Strips allowing an easier access for supernatant aspiration.

Add **24 µL of NucleoMag® B-Beads** and **320 µL of Binding Buffer IMB**. Mix by pipetting up and down 6 times and **shake** for **5 min** at **room temperature**. Alternatively, when processing the kit without a shaker, pipette up and down 10 times and incubate for 5 min at room temperature.

Be sure to resuspend the NucleoMag® B-Beads before removing them from the storage bottle. Vortex storage bottle briefly until a homogenous suspension has formed.

Separate the magnetic beads against the side of the wells by placing the Square-well Block on the NucleoMag® SEP magnetic separator. Wait at least **5 min** until all the beads have been attracted to the magnets. Remove and discard supernatant by pipetting.

Note: Do not disturb the attracted beads while aspirating the supernatant. The magnetic pellet might not be visible in this step. Remove supernatant from the opposite side of the well.

5 Proceed with step 5 of detailed protocol for isolation of DNA from insects (see page 4 of this support protocol).