Protocol for extraction of genomic DNA from INSECTS using the MagMAXCore Kit (KingFisher DuoPrime)

1. Materials/Reagents needed:

- MagMAX™ CORE Kit (P/N: A32700 – A32702)
- PK Buffer for MagMAX™-96 DNA Multi-Sample Kit (P/N: 4489111)
- Witeg ™ glass beads (P/N: 10450975 Fisher Scientific)
- PBS - Phosphate-Buffered Saline (10X) pH 7.4, RNase-free (P/N: AM9624 – AM9625)
- (OPTIONAL) DTT 1M (P/N: P2325).

2. Preparation of plates for KingFisher™ DuoPrime instrument:

ID	Row	Reagents	Volume/Well	Placa
Sample	A	Sample + reagents	See below	Deep Well Plate
Wash 1	B	MagMAX Core Wash Solution 1	500 µl	
Wash 2	C	MagMAX Core Wash Solution 2	500 µl	
Tip Comb	H	/	/	
Elution Strip	/	MagMAX Core Elution Buffer	90 µl	Elution Strip

3. Preparation of Lysis/Binding/Beads mix

Combine the following components for the required number of samples plus 10% overage.

Component	Volume per sample
MagMAX CORE Lysis Solution	350 µl
MagMAX CORE Binding Solution	350 µl
MagMAX CORE Magnetic Beads	20 µl
Final volume	**720 µl**

Comment: Mix Magnetic Binding Beads thoroughly before pipetting. Prepare fresh solution before use.

4. Preparation of PK / PK Buffer (DTT) mix

Combine the following components for the required number of samples plus 10% overage.

Component	Volumen por muestra
PK Buffer for MagMAX™-96 DNA Multi-Sample Kit	90 µl
MagMAX CORE Proteinase K	10 µl
(DTT 1M – Optional)	(5 µl)
Final Volume	**100 (105) µl**

The amount of nucleic acid extracted can vary considerably depending on the method.

Workflow 1 – Glass beads

1. Transfer up to 50 mg of insect tissue to a 1.5ml microcentrifuge tube (Eppendorf).
2. Add 2-3 glass beads to grind the tissue in a homogenizer (see Appendix 1).
3. Add 100 µl (105 µl) of PK/PK buffer mix to the destroyed tissue (if tissue is not completely covered by the liquid, add 100 µl of 1x PBS or add another 100 µl of PK/PK buffer mix).
4. Vortex and incubate at 60°C for 1-3 hours or until tissue is completely dissolved (if material remains after incubation, centrifuge for 2 minutes at 14,000xg and then transfer supernatant).
5. Transfer the lysate (step 4) into the wells of row A of a Deep Well plate.
6. Resuspend well and add 720 µL of the Lysis/Binding/Bead mix to the lysates (row A of deep plate).
7. Load the plate on the machine and start the *MagMAX_CORE_DUO.bdz* protocol.

Workflow 2 - Mortar

1. Transfer up to 50 mg of insect tissue to a mortar.
2. Add liquid nitrogen (or other reagent) to the mortar and grind the tissue thoroughly with a pestle.
3. Transfer the tissue powder to a 1.5 ml microcentrifuge tube.
4. Add 100 µl (105 µl) of PK/PK buffer mixture to the grinded tissue (if the tissue is not completely covered by the liquid, add 100 µl of 1X PBS or add another 100 µl of PK/PK Buffer mixture).
5. Vortex and incubate at 60°C for 1-3 hours or until tissue is completely dissolved (if material remains after incubation, centrifuge for 2 minutes at 14,000xg and then transfer supernatant).
6. Transfer the lysate (step 5) into the wells of row A of a Deep Well plate.
7. Resuspend well and add 720 µL of the Lysis/Binding/Bead mix to the lysates (row A of deep plate).
8. Load the plate on the machine and start the *MagMAX_CORE_DUO.bdz* protocol

Workflow 3 - Insect Legs

1. Remove the legs of the insect (2-4 legs) using tweezers (if you can, also remove some tissue (at the body attachment)).
2. Transfer the paws to a 1.5 ml microcentrifuge tube.
3. Manually grind the paws and add 100 µl (105 µl) of PK/PK buffer mixture to the grinded tissue + 100 µl of 1X PBS (make sure the paws are completely covered by the liquid; if the tissue is not completely covered for the liquid, add 100 µl of 1X PBS or add another 100 µl of PK/PK Buffer mixture).
4. Vortex and incubate at 60°C for 1-3 hours or until tissue is completely destroyed (if material remains after incubation, centrifuge for 2 minutes at 14,000xg and then transfer supernatant).
5. Transfer the lysate (step 4) into the wells of row A of a Deep Well plate.
6. Resuspend well and add 720 µL of the Lysis/Binding/Bead mix to the lysates (row A of deep plate).
7. Load the plate on the machine and start the *MagMAX_CORE_DUO.bdz* protocol

Workflow 4 - Whole insect (without grinding the insect) or Head (from 1mm to more than 5mm)

1. Place the whole insect/head into a microcentrifuge tube large enough to contain it.
2. A) For the Head: Add 100 µl (105 µl) of PK/PK buffer mixture to the tissue + 100 µl of 1X PBS (make sure the head is completely covered by the liquid; if it is not completely covered by the liquid, add another 1X PBS or add another 100 µl of PK/PK Buffer mix).
   B) For the whole Insect: Add 100 µl (105 µl) of PK/PK buffer mixture to the tissue + 200 µl of 1X PBS (make sure the insect is completely covered by the liquid; if it is not completely covered by the liquid, add another 100 µl of 1X PBS or add another 100 ul of PK/PK Buffer mix).
3. Mix and incubate at 56ºC for 16-18 hours (overnight).
4. Collect the entire available volume (max 300 µl) and transfer it into the wells of row A of a Deep Well plate.
5. Resuspend well and add 720 µL of the Lysis/Binding/Bead mix to the lysates (row A of deep plate).
6. Load the plate on the machine and start the *MagMAX_CORE_DUO.bdz* protocol

Appendix 1.

Homogenizers and indicative speeds

Option	Settings
Fisher Scientific™ Bead Mill 24 Homogenizer	6 m/s; 3 minutes
Mixer Mill 400	30 Hz; 10 minutes
Precellys™ 24 Homogenizer	6,800 rpm; 2 × 90 seconds
FastPrep-24™ Instrument	6.5 M/s; 4 × 45 seconds
Mini-BeadBeater-96	5 minutes