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HighPrep Insect DNA Kit

Isolation of gDNA from a Variety of Insects

Catalog Nos. HPI-D5, HPI-D96, HPI-D96x4
Manual Revision 3
WI-72-21

- Genomic DNA isolation from insects
- Magnetic bead-based chemistry

Protocol

Contents

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Product Description

The HighPrep Insect DNA Kit is a high quality genomic DNA purification kit. This kit can be used for a variety of insects, including mosquitoes. Up to 96 insect samples can be processed in less than an hour. The kit utilizes our proprietary magnetic beads chemistry and requires no phenol or chloroform extraction or alcohol precipitation and is suited for high throughput automation. The purified high quality genomic DNA is suitable for direct use in most downstream applications such as amplification and enzymatic reactions.

Process

The HighPrep Insect DNA Kit uses a simple 3 step procedure: Lyse+Bind-Wash-Elute. Samples are lysed and DNA binds to the MAG-S1 Particles in one step. Utilizing a magnetic separation device, the bound genomic DNA is separated from the solution and is washed. The final step is elution of high quality genomic DNA from the magnetic beads.

Kit Contents and Storage

HighPrep Insect DNA Kit Catalog No.	**HPI-D5 (Sample)**	**HPI-D96**	**HPI-D96x4**	**Storage**
Number of Preps	**5**	**96**	**384**	
MAS Buffer	2 mL	33 mL	125 mL	15-25°C
MTS Buffer	2.5 mL	40 mL	160 mL	15-25°C
HSW Buffer[1]	1.6 mL	22 mL	88 mL	15-25°C
MB Elution Buffer	1 mL	40 mL	120 mL	15-25°C
Pro K Solution[2]	125 µL	2.5 mL	10 mL	2-8°C
MAG-S1 Particles[3]	55 µL	1.1 mL	4.4 mL	2-8°C

[1]Ethanol must be added prior to use. See preparation of reagents section.
*Once opened, reagents are usable until the expiration date on the product label. Be sure to close the lid firmly before storing reagents for later use.

Shipping and Storage

- [2] Pro K Solution comes in a ready to use solution. Ships at room temperature. Store at 2-8°C.
- [3]MAG-S1 Particles ship at room temperature. Store at 2-8°C.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). The SDS can be downloaded from the "Product Documents" tab when viewing this product at www.magbiogenomics.com.

Preparation of Reagents

Prepare the following components for each kit before use:

Catalog No.	Component	Add 100% Ethanol*	Storage
HPI-D5 (Sample)	HSW Buffer	1.46 mL	Room Temp 15-25°C

Catalog No.	Component	Add 100% Ethanol*	Storage
HPI-D96	HSW Buffer	28 mL	Room Temp 15-25°C

Catalog No.	Component	Add 100% Ethanol*	Storage
HPI-D96x4	HSW Buffer	112 mL	Room Temp 15-25°C

**Ensure bottle/tube lid Is closed tightly when preparing and storing reagents.*

Amounts of Starting Material

Use the amounts of starting material indicated in the following table.

Sample	Amount
Insects	4 to 6 mm

Protocol: Insects (1.5 mL tube or 96 well format)

Equipment and Reagents to Be Supplied by the User

- ☐ 96 well round-bottom deep well plates with a capacity of 1 mL per well. (ABgene AB1127)
- ☐ Magnetic separation device compatible with 1.5 mL centrifuge tube and 96-well plate
- ☐ Centrifuge with swing bucket rotor capable of 4,000 x g
- ☐ Bench top microcentrifuge
- ☐ Shaking water bath
- ☐ Razor Blade
- ☐ Spatula
- ☐ PTFE Tissue Grinder stick (VWR 89026-404)
- ☐ Weigh Dish, Medium size (31/2x3 1/2x)
- ☐ Vortexer
- ☐ 70% Ethanol
- ☐ 100% Ethanol
- ☐ Beta-Mercaptoethanol
- ☐ Optional RNase A (10 mg/mL)

Things to do Before Starting

- ☐ Equilibrate samples to room temperature
- ☐ Ensure HSW Buffer is prepared according to the instructions on page 2
- ☐ AS Buffer may show precipitates during storage. If precipitates are present, heat bottle to 37°C to dissolve the precipitates before use

Protocol

⚠ Bring the **MAG-S1 Particles** to room temperature for at least 30 minutes before use.

1. Add 4-6 mm of insect into a weighing dish.

2. Cut/mince into smaller pieces

   a. Legs and Wings: Mince led and wings in smallest pieces possible.

   Optional: To improve lysis and reduce incubation time, pulverize sample to fine powder in liquid nitrogen.

3. Add 500 µL of **MTS Buffer**.

   a. Legs and Wings: Add 50 µL of beta-mercaptoethanol.

4. Grind insect parts using PTFE Tissue Grinder stick.

5. Transfer ground insect parts into a 1.5 mL microcentrifuge tube or in the plate well.

6. Add 20 µl of **Pro K Solution**. Vortex to mix well and incubate at 65°C in a shaking water bath for 30 minutes. Resuspend the mixture once during lysis.

   a. Legs and Wings**:** Add 30 µL of **Pro K Solution**. Vortex to mix well and incubate at 65°C in a shaking water bath for 3-4 hours. Resuspend the mixture every 20 minutes during lysis if a shaking water bath is not available.

7. Centrifuge the tube or plate at maximum speed for 5 minutes to pellet the undigested materials.

8. Transfer the clear lysate (as much as possible without adding any undigested material) to a new processing tube or plate (well must have capacity of 400 µL or more).
Optional: RNA in the sample will be copurified. If the RNA will interfere with your downsteam application, remove the RNA by adding 5 µL of RNase A. Pipette mix for 20 times or vortex for 15 seconds.

9. Add 200 µL of **MAS Buffer** to the sample and pipette mix 20 times or vortex for 15 seconds. Incubate the sample plate at 65°C for 10 minutes.

10. Bring the sample plate to room temperature and add 290 µL of 100% Ethanol and 10 µL of **MAG-S1 Particles** to the sample, and pipette mix 20 times. Incubate the sample plate at room temperature for 5 minutes.

    ⚠ *Shake well to resuspend the **MAG-S1 Particles** before use.*

11. Place the sample processing plate containing the sample on the magnetic separation device for 3 minutes to magnetize the **MAG-S1 Particles**.

12. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

13. Remove the plate from the magnetic separation device, add 400 µL of **HSW Buffer** to the sample and mix by pipetting 25 times or vortex for 1 minute to resuspend the **MAG-S1 Particles**.

    ⚠ *Complete resuspension of the **MAG-S1 Particles** is crucial for obtaining high purity.*

14. Place the sample plate back on the magnetic separation device and wait 5 minutes or until the magnetic beads clear from the solution.

15. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

16. Remove the plate from the magnetic separation device, add 400 µL of 70% Ethanol to the sample and mix by pipetting 25 times or vortex for 1 minute to resuspend the **MAG-S1 Particles**. Incubate at room temperature for 3 minutes.

17. Place the sample processing plate containing the sample on the magnetic separation device for 3 minutes to magnetize the **MAG-S1 Particles**.

18. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

19. Repeat steps 16-18 for a second Ethanol wash.

20. Dry the beads by incubating for 10 minutes at room temperature with the plate still on the magnetic separation device.

    ⚠ *Do not overdry the beads.*

21. Remove the plate from the magnetic separation device. Add 50-200 µL of **MB Elution Buffer** or nuclease-free water to the sample and mix 50 times or vortex for 2 minutes to completely resuspend the **MAG-S1 Particles.**

    a. Leg and wings**:** Use 50 µL of **MB Elution Buffer** for elution**.**

    ⚠ *Complete resuspension of the **MAG-S1 Particles** is crucial for obtaining high purity.*

22. Incubate at room temperature for 10 minutes.

23. Place the sample plate back on the magnetic separation device and wait 5 minutes or until the magnetic beads clear from the solution.

24. Transfer the eluate (cleared supernatant containing the DNA) to a microplate for storage. Store the DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via:
Phone: US/Canada, +1 301-302-0144. Europe, +49 7250 33 13 403
Email: US/Canada, support@magbiogenomics.com. Europe, info.europe@magbiogenomics.com

Symptoms	Possible Causes	Comments
Low DNA yield	Incomplete resuspension of the MAG-S1 Particles	Resuspend the MAG-S1 Particles by vortexing vigorously before use
	Loss of MAG-S1 Particles during operation	Avoid disturbing the MAG-S1 Particles during aspiration of supernatant
	DNA remains bound to the MAG-S1 Particles	Increase elution volume and incubate for 15 minutes. Pipette mix 50 to 100 times
	Ethanol is not added into HSW Buffer	Add absolute 100% Ethanol to the HSW Buffer (see page 2 for instructions)
MAG-S1 Particles do not completely clear from the solution	Magnetizing time too short	Increase collection time on the magnet
Problems in downstream applications	Insufficient DNA in starting material	Use more starting material
	Ethanol carry-over	Dry the MAG-S1 Particles completely before elution

Ordering Information

HighPrep Insect DNA Kit

Catalog No.	Product	Description	Preps
HPI-D96	HighPrep Insect DNA Kit (96 preps)	Magnetic bead-based kit for Genomic DNA isolation from 4-6 mm of insects.	96
HPI-D96x4	HighPrep Insect DNA Kit (384 preps)		384

Related Products

HighPrep PCR

Catalog No.	Product
AC-60005	HighPrep PCR (5 mL)
AC-60050	HighPrep PCR (50 mL)
AC-60250	HighPrep PCR (250 mL)
AC-60500	HighPrep PCR (500 mL)

Magnetic Separation Devices

Catalog No.	Product
MYMAG-96	Handheld Magnetic Separation Device (96 well microplate format)
MYMAG-96X	Magnetic Separation Device (96 well ring format)
MBMS-12	MagStrip Magnet Stand (1.5 mL x 12)
MBMS-31550	15 mL and 50 mL Magnetic Stand Combo (3 x 15 mL and 3 x 50 mL)

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