Liquid Biopsy Research - “We are where you start”

HighPrep™ Insect DNA Kit

Manual Revision v1.1
Catalog Nos. HPI-D16, HPI-D96, HPI-D96X4

- Genomic DNA isolation from Insects such as mosquitoes
- Magnetic beads based chemistry

PROTOCOL

Contents

Product Description and Process.. 1
Kit Contents, Storage, Stability.. 1
Preparation of Reagents 2
Mosquito DNA 1.5mL tube or 96 well protocol............... 3
Troubleshooting Guide.. 6

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Product Description

The HighPrep™ Insect DNA Kit is a high quality genomic purification kit for a variety of Insect sources including: Mosquitoes. Up to 96 samples of tissues 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 magnetic beads 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-D16	HPI-D96	HPI-D96X4	STORAGE
Number of Preps	**16**	**96**	**384**	
MAS Buffer	6 ml	33 ml	125 ml	15-25°C
MTS Buffer	8 ml	40 ml	160 ml	15-25°C
HSW Buffer [1]	5.5 ml	22 ml	88 ml	15-25°C
MB Elution Buffer	8 ml	40 ml	120 ml	15-25°C
Pro K Solution [2]	500 µl	2.5 ml	10 ml	2-8°C
MAG-S1 Particles	250 µl	1.1 ml	4.4 ml	2-8°C

[1] Ethanol must be added prior to use. See Preparation of Reagents

Stability

All components are stable for 12 months when stored accordingly.

[2] Pro K Solution comes in a ready to use solution. Component is stable for 1 year when stored at 15-25°C. For storage longer than 1 year, storage at 2-8°C is recommended.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). MSDS can be downloaded from the "Product Resource" tab when viewing the product kit.

Preparation of Reagents

Prepare the following components for each kit before use:

Catalog No.	Component	Add 100% Ethanol	Storage
HPI-D16	HSW Buffer	7 ml	Room Temp 15-25°C
Components are stable for 1 year when stored closed at room temperature			

Catalog No.	Component	Add 100% Ethanol	Storage
HPI-D96	HSW Buffer	28 ml	Room Temp 15-25°C
Components are stable for 1 year when stored closed at room temperature			

Catalog No.	Component	Add 100% Ethanol	Storage
HPI-D96X4	HSW Buffer	112 ml	Room Temp 15-25°C
Components are stable for 1 year when stored closed at room temperature			

Amounts of starting material

Use the amounts of starting material indicated in Table 1.

Sample	Amount
Mosquitoes	4 to 6

HighPrep™ Insect DNA Kit : Mosquito- 96 well and 1.5 ml tube format

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- ☐ 96 well round-bottom deep well plates with a capacity of 1 mL per well. (ABgene AB-1127)
- ☐ Magnetic separation device compatible with 1.5mL 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 and is at room temperature.
AS Buffer may show precipitates during storage. If precipitates are present, heat bottle to 37°C to dissolve the precipitates before use.

Protocol

1. **Add Take 4-6 mm mosquitos 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 500µl MTS Buffer.**

   a. ***Legs and Wings*: Add 50ul beta-mercaptoethanol**

4. **Grind mosquito parts using PTFE Tissue Grinder stick.**

5. **Transfer ground mosquito parts tissues into 1.5 mL micro centrifuge tube or in the well.**

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

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

7. **Centrifuge the tube or plate at maximum speed for 5 min 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 mouse tail will be copurified. If the RNA will interfere with your downsteam application, remove the RNA by adding 5 µl RNase A. Pipette mix for 20 times or vortex for 15 sec.

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

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

    ⚠ Shake thoroughly the MAG-S1 particles to fully resuspend before use.

11. **Place the sample processing plate containing the sample on the magnetic separation device for 3 min 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 HSW Buffer to the sample and mix by pipetting 25 times or vortex for 1 min to resuspend the MAG-S1 particles.**

    ⚠ Complete resuspension of the MAG-S1 particles is crucial for obtaining purity.

14. **Place the sample plate back on the magnetic separation device and wait 5 min or until the magnetic beads clear from 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 off the magnetic separation device, add 400 µL 70% ethanol to the sample and mix by pipetting 25 times or vortex for 1 min to resuspend the MAG-S1 particles. Incubate at room temperature for 3 min.**

17. **Place the sample processing plate containing the sample on the magnetic separation device for 3 min 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 to 18 for a second ethanol wash.

20. Dry the beads by incubating for 10 min 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 MB Elution Buffer or nuclease-free water to the sample and mix 50 times or vortex for 2 min to completely resuspend the MAG-S1 particles.

a. *Leg and wings*: Use 50 ul 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 min.

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

24. Transfer the eluate (cleared supernatant containing the DNA) to a microplate for storage. Store 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: 1-855-262-4246 (in US), outside US, 1-301-302-0144
Email: support@magbiogenomics.com

Symptoms	Possible Causes	Comments
Low DNA Yields	Incomplete resuspension of MAG-S1 particles	Resuspend 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. Pipet mix 50 to 100 times.
	Ethanol is not added into HSW Buffer	Add absolute 100% Ethanol to HSW Buffer (see page 2 for instructions).
MAG-S1 particles do not completely clear from solution	Too short of magnetizing time	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

Product Description	Catalog No.	Preps
HighPrep™ Insect DNA Kit	HPI-D96	96
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