HighPrep HMW DNA Kit

Isolation of High Molecular Weight DNA

Catalog Nos. HPHMW-D5, HPHMW-D96, HPHMW-D96x4
Manual Revision 2
WI-72-127

- High molecular weight DNA isolation from whole blood, bone marrow, saliva, buccal cells, cultured cells, tissues, and bacteria
- Magnetic bead-based chemistry

Protocol

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Product Description

The HighPrep High Molecular Weight DNA Kit is specifically designed for extraction of high molecular weight DNA (HMW DNA) in the size range of 50–300+ kb. The kit extracts HMW DNA from biological samples such as whole blood, bone marrow, saliva, buccal cells, cultured cells, tissues, and bacteria. The HighPrep High Molecular Weight DNA Kit utilizes magnetic bead-based technology in combination with chaotropic agents to gently isolate HMW DNA and remove inhibitors. The HMW DNA purified this way is particularly suitable for analysis on long read sequencing genomic platforms including PacBio RSII/Sequel/Sequel II and Oxford Nanopore.

DNA purified using the HighPrep High Molecular Weight DNA Kit is of good quality and purity. The kit includes RNase A for the removal of RNA. The exact size of extracted DNA varies depending on sample matrix, the quality of the starting material, and processing conditions.

Deviating from the recommended protocol, particularly the pipette-mixing or vortex instructions, will cause mechanical shearing of DNA and decrease average fragment sizes. To maximize genomic DNA size, the protocols for this kit do not incorporate pipette mixing or vortex mixing of the sample during the extraction procedures, except during the elution step.

Features

- Superior DNA yield and high quality DNA from different sample matrices
- Simplified, user friendly protocols that remove RNA and inhibitors
- Reproducible high molecular weight DNA isolation (50-300+ kb)
- Automation friendly and can be easily scaled up
- Great performance in next generation sequencing, third generation sequencing, genotyping, PCR, restriction enzyme digestion, and cloning

Kit Contents and Storage

HighPrep HMW DNA Kit Catalog No.	HPHMW-D5	HPHMW-D96	HPHMW-D96x4	Storage
Number of Preps	**5**	**96**	**384**	
HAS Buffer	2 mL	30 mL	120 mL	15-25°C
HTS Buffer	1.8 mL	25 mL	100 mL	15-25°C
HMW1 Buffer[1]	3 mL	50 mL	200 mL	15-25°C
HMW2 Buffer[1]	2 mL	30 mL	120 mL	15-25°C
MB Elution Buffer	1.2 mL	20 mL	80 mL	15-25°C
MAG-HM1 Particles	55 µL	1 mL	4 mL	15-25°C
Pro K Solution[2]	125 µL	2 mL	8 mL	2-8°C
RNase A[3]	30 µL	500 µL	2 mL	2-8°C

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

Shipping and Storage

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

Safety Information

Any consumables, including plates, tubes, etc., used to process samples with infectious or microbial hazards should be disposed of in an appropriate biohazard waste bin. 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
HPHMW-D5	HMW1 Buffer	3 mL	Room Temp 15-25°C
	HMW2 Buffer	5 mL	

Catalog No.	Component	Add 100% Ethanol*	Storage
HPHMW-D96	HMW1 Buffer	50 mL	Room Temp 15-25°C
	HMW2 Buffer	75 mL	

Catalog No.	Component	Add 100% Ethanol*	Storage
HPHMW-D96x4	HMW1 Buffer	200 mL	Room Temp 15-25°C
	HMW2 Buffer	300 mL	

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

Protocol: HMW DNA Extraction from Gram Negative Bacteria

Equipment and Reagents to Be Supplied by the User

Single tube format

- ☐ 1.5 mL Protein LoBind microcentrifuge tubes (reduces protein contamination and improves DNA purity)
- ☐ Magnetic Rack Separator for 1.5 mL microcentrifuge tube (MagStrip Magnet Stand (Cat# MBMS-12)
- ☐ Wide-bore pipette tips (200 µL and 1000 µL pipette tips with aerosol barrier)
- ☐ Mini-tube rotator
- ☐ Microcentrifuge (with rotor for 1.5 mL-2 mL tubes)
- ☐ Minicentrifuge
- ☐ Vortexer
- ☐ Water bath, thermomixer, or heat block capable of 55°C
- ☐ 100% Ethanol
- ☐ 70% Ethanol
- ☐ 1X PBS

Things to do Before Starting

- ☐ Ensure that HMW1 Buffer and HMW2 Buffer are prepared according to the Preparation of Reagents section on page 2.
- ☐ If performing manual DNA extraction, preset water bath, thermomixer, or heating blocks to 55°C.
- ☐ HMW1 Buffer may show precipitates during storage. If precipitates are present, heat the bottle to 37°C before use.
- ☐ Make sure to resuspend the MAG-HM1 Particles by vortexing before use.

Processing Tips

- ☐ Avoid repeated freeze thaw of samples and store samples properly to avoid DNA shearing.
- ☐ Use low bind tubes to prevent DNA from binding to the tube walls.
- ☐ Use slow, gentle pipetting technique to help keep DNA intact during extraction.
- ☐ To avoid DNA shearing, use wide-bore pipette tips and avoid vortexing.

Protocol

Cell input requirements: 1 mL of culture with OD600 reading of 1 or near 1. Overloading bacterial cells will lead to inefficient lysis and poor DNA purity.

- For bacteria with >1 OD readings, consider scaling up extraction reagents or diluting the sample.

1. Harvest bacterial cells by centrifuging the culture at 16,000 x g for 1 minute at 4°C. The pellet can be processed as a fresh pellet or frozen. Use Protein LoBind tubes for culture centrifugation.

2. Discard the culture supernatant and add 180 µL of 1X PBS. Pipette the sample up and down to resuspend the bacterial pellet in PBS. Make sure there are no visible cell lumps and the sample looks homogenous.

3. Add 20 µL of **Pro K Solution** and 200 µL of **HAS Buffer** to the sample. Pulse vortex for 1 second x 5 times (max setting). Incubate at 55°C in a water bath (needs periodic agitation-invert the tube at least 3-5 times during incubation) or incubate in a thermomixer for 30 minutes (900 rpm).

4. Bring the sample to room temperature and add 5 uL of **RNase A**. Pulse vortex for 1 second x 5 times (max setting). Incubate the sample at room temperature for 3 minutes.

5. Incubate the sample at 70°C in a water bath (need periodic agitation) or thermomixer (2000 rpm) for 10 minutes.

6. Add 290 uL of 100% Ethanol and 10 uL of **MAG-HM1 Particles** to the sample. Pulse vortex for 1 second x 5 times (max setting).

   ⚠ *Shake well to resuspend the **MAG-HM1 Particles** before use.*

7. Place the sample on a mini-rotator and rotate the samples at room temperature for 10 minutes. It is normal for lumps of beads and DNA to form during rotation. Do not break up the lumps. Briefly spin the tube on a minicentrifuge for 2 seconds.

8. Place the sample on a magnetic separation device for 5 minutes or until the beads are completely cleared from the solution. Discard the supernatant by pipetting the liquid out. Make sure to remove any residual liquid from the tube cap.

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

9. Remove the sample from the magnetic separation device.

10. Add 500 µL of **HMW1 Buffer** and put the sample on a mini-rotator. Gently rotate at room temperature for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds.

11. Place the sample on a magnetic separation device for 5 minutes or until the beads are completely cleared from the solution. Discard the supernatant by pipetting. Make sure to remove any residual liquid from the tube cap.

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

12. Remove the sample from the magnetic separation device.

13. Repeat steps 10-12 for a second **HMW1 Buffer** wash.

14. Add 500 µL of **HMW2 Buffer** and put the sample on the mini-rotator. Gently rotate at room temperature for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds.

15. Place the sample on a magnetic separation device for 5 minutes or until the beads are completely cleared from the solution. Discard the supernatant by pipetting the liquid out. Make sure to remove the any liquid from the tube cap.

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

16. Remove the sample from the magnetic separation device.

17. Repeat steps 14-15 for a second **HMW2 Buffer** wash.

18. Keep the sample on the magnetic separation device and air dry the magnetic beads at room temperature for 5 minutes. Remove any residual liquid with a pipette.

    ⚠ *It is critical to completely remove all liquid from the tube.*

19. Add 50-100 µL of **MB Elution Buffer** to the sample. Do not use a pipette or vortex to mix the sample. First, incubate the sample at 55°C in a thermomixer (2000 rpm) for 5 minutes. Then, remove the sample from 55°C incubation and gently mix the sample by pulse vortexing for 1 second. Last, incubate the sample at 55°C for an additional 5 minutes.

20. Place the sample back on the magnetic separation device for 5 minutes or until the **MAG-HM1 Particles** are completely cleared from the **MB Elution Buffer**.

21. Transfer the eluate (cleared supernatant) to a new 1.5 mL microcentrifuge tube and keep at 4°C for subsequent applications. For long term storage, keep the DNA at -20°C.

    ⚠ *Do not freeze and thaw HMW DNA repeatedly. This will break the DNA into smaller pieces.*

Protocol: HMW DNA Extraction from Gram Positive Bacteria

Equipment and Reagents to Be Supplied by the User

Single tube format

- ☐ 1.5 mL Protein LoBind microcentrifuge tubes (reduces protein contamination and improves DNA purity)
- ☐ Magnetic Rack Separator for 1.5 mL microcentrifuge tube (MagStrip Magnet Stand (Cat# MBMS-12)
- ☐ Wide-bore pipette tips (200 µL and 1000 µL pipette tips with aerosol barrier)
- ☐ Mini-tube rotator
- ☐ Microcentrifuge (with rotor for 1.5 mL-2 mL tubes)
- ☐ Minicentrifuge
- ☐ Vortexer
- ☐ Water bath, thermomixer, or heat block capable of 55°C
- ☐ 100% Ethanol
- ☐ 70% Ethanol
- ☐ Lysozyme stock solution (100 mg/mL)
- ☐ 1X PBS

Things to do Before Starting

- ☐ Ensure that HMW1 Buffer and HMW2 Buffer are prepared according to the Preparation of Reagents section on page 2.
- ☐ If performing manual DNA extraction, preset water bath, thermomixer, or heating blocks to 55°C.
- ☐ HMW1 Buffer may show precipitates during storage. If precipitates are present, heat the bottle to 37°C before use.
- ☐ Make sure to resuspend the MAG-HM1 Particles by vortexing before use.

Processing Tips

- ☐ Avoid repeated freeze thaw of samples and store samples properly to avoid DNA shearing.
- ☐ Use low bind tubes to prevent DNA from binding to the tube walls.
- ☐ Use slow, gentle pipetting technique to help keep DNA intact during extraction.
- ☐ To avoid DNA shearing, use wide-bore pipette tips and avoid vortexing.

Protocol

Cell input requirements: 1 mL of culture with OD600 reading of 1 or near 1. Overloading bacterial cells will lead to inefficient lysis and poor DNA purity.

- For bacteria with >1 OD readings, consider scaling up extraction reagents or dilutiing the sample.

1. Harvest bacterial cells by centrifuging the culture at 16,000 x g for 1 minute at 4°C. The pellet can be processed as a fresh pellet or frozen. Use Protein LoBind tubes for culture centrifugation.

2. Discard the culture supernatant and add 130 µL of 1X PBS. Pipette the sample up and down to resuspend the bacterial pellet in PBS. Make sure there are no visible cell lumps and the sample looks homogenous.

3. Add 50 µL of Lysozyme (100 mg/mL) and mix by tapping the tube.

4. Incubate the sample in a thermomixer for 30 minutes (900 rpm) at 37°C.

5. Add 20 µL of **Pro K Solution** and 200 µL of **HAS Buffer**, pulse vortex for 1 second (max setting), and incubate at 55°C in a water bath (needs periodic agitation-invert the tube at least 3-5 times during incubation) or in a thermomixer for 10 minutes (2000 rpm).

6. Bring the sample to room temperature and add 5 uL of **RNase A**. Pulse vortex for 1 second x 5 times (max setting). Incubate the sample at room temperature for 3 minutes.

7. Incubate the sample at 70°C in a water bath (need periodic agitation) or thermomixer (2000 rpm) for 10 minutes.

8. Add 290 uL of 100% Ethanol and 10 uL of **MAG-HM1 Particles** to the sample. Pulse vortex for 1 second x 5 times (max setting).

9. Place the sample on a mini-rotator and rotate the samples at room temperature for 10 minutes. It is normal for lumps of beads and DNA to form during rotation. Do not break up the lumps. Briefly spin the tube on a minicentrifuge for 2 seconds.

10. Place the sample on a magnetic separation device for 5 minutes or until the beads are completely cleared from the solution. Discard the supernatant by pipetting. Make sure to remove any residual liquid from the tube cap.

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

11. Remove the sample from the magnetic separation device.

12. Add 500 µL of **HMW1 Buffer** and put the sample on the mini-rotator. Gently rotate at room temperature for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds.

13. Place the sample on a magnetic separation device for 5 minutes or until the beads are completely cleared from the solution. Discard the supernatant by pipetting the liquid out. Make sure to remove any residual liquid from the tube cap.

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

14. Remove the sample from the magnetic separation device.

15. Repeat steps 12-14 for a second **HMW1 Buffer** wash.

16. Add 500 µL of **HMW2 Buffer** and put the sample on the mini-rotator. Gently rotate at room temperature for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds.

17. Place the sample on a magnetic separation device for 5 minutes or until the beads are completely cleared from the solution. Discard the supernatant. Make sure to remove any residual liquid from the tube cap.

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

18. Remove the sample from the magnetic separation device.

19. Repeat steps 16-17 for a second **HMW2 Buffer** wash.

20. Keep the sample on the magnetic separation device and air dry the magnetic beads at room temperature for 5 minutes. Remove any residual liquid with a pipette.

    ⚠ *It is critical to completely remove all liquid from the tube.*

21. Add 50-100 µL of **MB Elution Buffer** to the sample. Do not use a pipette or vortex to mix the sample. First, incubate the sample at 55°C in a thermomixer (2000 rpm) for 5 minutes. Then, remove the sample from 55°C incubation and gently mix the sample by pulse vortexing for 1 second. Last, incubate the sample at 55°C for an additional 5 minutes.

22. Place the sample back on the magnetic separation device for 5 minutes or until the **MAG-HM1 Particles** are completely cleared from the **MB Elution Buffer**.

23. Transfer the eluate (cleared supernatant) to a new 1.5 mL microcentrifuge tube and keep at 4°C or for subsequent applications. For long term storage, keep the DNA at -20°C.

    ⚠ *Do not freeze and thaw HMW DNA repeatedly. This will break the DNA into smaller pieces.*

Protocol: HMW DNA Extraction from Buccal Cells or Cultured Cells

Equipment and Reagents to Be Supplied by the User

Single tube format

- ☐ 1.5 mL Protein LoBind microcentrifuge tubes (reduces protein contamination and improves DNA purity)
- ☐ Magnetic Rack Separator for 1.5 mL microcentrifuge tube (MagStrip Magnet Stand (Cat# MBMS-12)
- ☐ Wide-bore pipette tips (200 µL and 1000 µL pipette tips with aerosol barrier)
- ☐ Mini-tube rotator
- ☐ Microcentrifuge (with rotor for 1.5 mL-2 mL tubes)
- ☐ Minicentrifuge
- ☐ Vortexer
- ☐ Water bath, thermomixer, or heat block capable of 55°C
- ☐ 100% Ethanol
- ☐ 70% Ethanol
- ☐ Optional: phosphate-buffered saline (PBS) or nuclease-free water may be required

Things to do Before Starting

- ☐ Ensure that HMW1 Buffer and HMW2 Buffer are prepared according to the Preparation of Reagents section on page 2.
- ☐ If performing manual DNA extraction, preset water bath, thermomixer, or heating blocks to 55°C.
- ☐ HMW1 Buffer may show precipitates during storage. If precipitates are present, heat the bottle to 37°C before use.
- ☐ Make sure to resuspend the MAG-HM1 Particles by vortexing before use.

Processing Tips

- ☐ Avoid repeated freeze thaw of samples and store samples properly to avoid DNA shearing.
- ☐ Use low bind tubes to prevent DNA from binding to the tube walls.
- ☐ Use slow, gentle pipetting technique to help keep DNA intact during extraction.
- ☐ To avoid DNA shearing, use wide-bore pipette tips and avoid vortexing.

Protocol

1. Centrifuge the sample at 2000 rpm for 5 minutes and remove the liquid medium.

2. Add 200 µL of **HTS Buffer** to the sample pellet.

3. Add 20 µL of **Pro K Solution**. Pulse vortex for 1 second (max setting). Incubate at 55°C in a thermomixer (2000 rpm) or water bath (needs periodic agitation-invert the tube at least 3-5 times during incubation) for 20 minutes.

4. Centrifuge the sample at 3,000 x g for 10 minutes and transfer the lysate to a new 1.5 mL tube.

   ⚠ *Do not transfer cell debris.*

5. Add 200 µL of **HAS Buffer** to the sample. Pulse vortex for 1 second (max setting). Incubate at 70°C for 10 minutes in a thermomixer (2000 rpm).

6. Bring the sample to room temperature and add 5 µL of **RNase A**. Pulse vortex for 1 second (max setting) or by tapping the tube several times and incubate for 3 minutes at room temperature.

7. Add 435 uL of 100% Ethanol and 10 uL of **MAG-HM1 Particles** to the sample. Pulse vortex for 1 second x 5 times (max setting).

8. Place the sample on a mini-rotator and rotate the samples at room temperature for 10 minutes. It is normal for lumps of beads and DNA to form during rotation. Do not break up the lumps. Briefly spin the tube on a minicentrifuge for 2 seconds.

9. Place the sample on a magnetic separation device for 5 minutes or until the beads are completely cleared from the solution. Discard the supernatant by pipetting. Make sure to remove any residual liquid from the tube cap.

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

10. Remove the sample from the magnetic separation device.

11. Add 500 µL of **HMW1 Buffer** and put the sample on a mini-rotator. Gently rotate at room temperature for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds.

12. Place the sample on a magnetic separation device for 5 minutes or until the beads are completely cleared from the solution. Discard the supernatant by pipetting the liquid out. Make sure to remove any residual liquid from the tube cap.

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

13. Remove the sample from the magnetic separation device.

14. Repeat steps 10-12 for a second **HMW1 Buffer** wash.

15. Add 500 µL of **HMW2 Buffer** and put the sample on the mini-rotator. Gently rotate at room temperature for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds.

16. Place the sample on a magnetic separation device for 5 minutes or until the beads are completely cleared from the solution. Discard the supernatant by pipetting the liquid out. Make sure to remove any residual liquid from the tube cap.

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

17. Remove the sample from the magnetic separation device.

18. Repeat steps 14-15 for a second **HMW2 Buffer** wash.

19. Keep the sample on the magnetic separation device and air dry the magnetic beads at room temperature for 5 minutes. Remove any residual liquid with a pipette.

    ⚠ *It is critical to completely remove all liquid from the tube.*

20. Add 50-100 µL of **MB Elution Buffer** to the sample. Do not use a pipette or vortex to mix the sample. First, incubate the sample at 55°C in a thermomixer (2000 rpm) for 5 minutes. Then, remove the sample from 55°C incubation and gently mix the sample by pulse vortexing for 1 second. Last, incubate the sample at 55°C for an additional 5 minutes.

21. Place the sample back on the magnetic separation device for 5 minutes or until the **MAG-HM1 Particles** are completely cleared from the **MB Elution Buffer**.

22. Transfer the eluate (cleared supernatant) to a new 1.5 mL microcentrifuge tube and keep at 4°C for subsequent applications. For long term storage, keep the DNA at -20°C.

    ⚠ *Do not freeze and thaw HMW DNA repeatedly. This will break the DNA into smaller pieces.*

Protocol: HMW DNA Extraction from Saliva, Whole Blood, and Bone Marrow

Equipment and Reagents to Be Supplied by the User

Single tube format

- ☐ 1.5 mL Protein LoBind microcentrifuge tubes (reduces protein contamination and improves DNA purity)
- ☐ Magnetic Rack Separator for 1.5 mL microcentrifuge tube (MagStrip Magnet Stand (Cat# MBMS-12)
- ☐ Wide-bore pipette tips (200 µL and 1000 µL pipette tips with aerosol barrier)
- ☐ Mini-tube rotator
- ☐ Microcentrifuge (with rotor for 1.5 mL-2 mL tubes)
- ☐ Minicentrifuge
- ☐ Vortexer
- ☐ Water bath, thermomixer, or heat block capable of 55°C
- ☐ 100% Ethanol
- ☐ 70% Ethanol
- ☐ 1X PBS

Automation

For Kingfisher™ Flex script, contact support@magbiogenomics.com

- ☐ 1.2 mL 96 deep well plates
- ☐ Wide-bore pipette tips (200 µL and 1000 µL pipette tips with aerosol barrier)
- ☐ Microcentrifuge (with rotor for 1.5 mL-2 mL tubes)
- ☐ Minicentrifuge
- ☐ Vortexer
- ☐ 100% Ethanol
- ☐ 70% Ethanol
- ☐ 1X PBS

Things to do Before Starting

- ☐ Ensure that HMW1 Buffer and HMW2 Buffer are prepared according to the Preparation of Reagents section on page 2.
- ☐ If performing manual DNA extraction, preset water bath, thermomixer, or heating blocks to 55°C.
- ☐ HMW1 Buffer may show precipitates during storage. If precipitates are present, heat the bottle to 37°C before use.
- ☐ Make sure to resuspend the MAG-HM1 Particles by vortexing before use.

Processing Tips

- ☐ Avoid repeated freeze thaw of samples and store samples properly to avoid DNA shearing.
- ☐ Use low bind tubes to prevent DNA from binding to the tube walls.
- ☐ Use slow, gentle pipetting technique to help keep DNA intact during extraction.
- ☐ To avoid DNA shearing, use wide-bore pipette tips and avoid vortexing.

1. Add 20 µL of **Pro K Solution** to a 1.5 mL tube.

2. Add 100 µL of **MB Elution Buffer**, 200 µL of either saliva or whole blood, and 300 µL of **HAS Buffer**.

3. Pulse vortex for 1 second (max setting) or invert the tube 5–10 times to mix and incubate the sample at 55°C in a thermomixer or water bath (needs periodic agitation-invert the tube at least 3-5 times during incubation) for 20 minutes.

4. Bring the sample to room temperature and add 5 µL of **RNase A**. Incubate for 3 minutes.

5. Add 430 uL of 100% Ethanol and 10 uL of **MAG-HM1 Particles** to the sample. Pulse vortex for 1 second x 5 times (max setting).

6. Place the sample on a mini-rotator and rotate the samples at room temperature for 10 minutes. It is normal for lumps of beads and DNA to form during rotation. Do not break up the lumps. Briefly spin the tube on a minicentrifuge for 2 seconds.

7. Place the sample on a magnetic separation device for 5 minutes or until the beads are completely cleared from the solution. Discard the supernatant by pipetting. Make sure to remove any residual liquid from the tube cap.

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

8. Remove the sample from the magnetic separation device.

9. Add 500 µL of **HMW1 Buffer** and put sample on the mini-rotator. Gently rotate at room temperature for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds.

10. Place the sample on a magnetic separation device for 5 minutes or until the beads are completely cleared from the solution. Discard the supernatant by pipetting the liquid out. Make sure to remove any residual liquid from the tube cap.

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

11. Remove the sample from the magnetic separation device.

12. Repeat steps 9-11 for a second **HMW1 Buffer** wash.

13. Add 500 µL of **HMW2 Buffer** and put the sample on the mini-rotator. Gently rotate at room temperature for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds.

14. Place the sample on a magnetic separation device for 5 minutes or until the beads are completely cleared from the solution. Discard the supernatant by pipetting. Make sure to remove the any liquid from the tube cap.

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

15. Remove the sample from the magnetic separation device.

16. Repeat steps 13-14 for a second **HMW2 Buffer** wash.

17. Keep the sample on the magnetic separation device and air dry the magnetic beads at room temperature for 5 minutes. Remove any residual liquid with a pipette.

    ⚠ *It is critical to completely remove all liquid from the tube.*

18. Add 50-100 µL of **MB Elution Buffer** to the sample. Do not use a pipette or vortex to mix the sample. First, incubate the sample at 55°C in a thermomixer (2000 rpm) for 5 minutes. Then, remove the sample from 55°C incubation and gently mix the sample by pulse vortexing for 1 second. Last, incubate the sample at 55°C for an additional 5 minutes.

19. Place the sample back on the magnetic separation device for 5 minutes or until the **MAG-HM1 Particles** are completely cleared from the **MB Elution Buffer**.

20. Transfer the eluate (cleared supernatant) to a new 1.5 mL microcentrifuge tube and keep at 4°C or for subsequent applications. For long term storage, keep the DNA at -20°C.

    ⚠ *Do not freeze and thaw HMW DNA repeatedly. This will break the DNA into smaller pieces.*

Protocol: HMW DNA Extraction from Tissues

Sample input: This protocol is for a tissue sample of 10 mg or less. If more tissue is processed, then HTS Buffer and HAS Buffer must be scaled up accordingly.

Equipment and Reagents to Be Supplied by the User

Single tube format

- ☐ 1.5 mL Protein LoBind microcentrifuge tubes (reduces protein contamination and improves DNA purity)
- ☐ Magnetic Rack Separator for 1.5 mL microcentrifuge tube (MagStrip Magnet Stand (Cat# MBMS-12)
- ☐ Wide-bore pipette tips (200 µL and 1000 µL pipette tips with aerosol barrier)
- ☐ Mini-tube rotator
- ☐ Microcentrifuge (with rotor for 1.5 mL-2 mL tubes)
- ☐ Minicentrifuge
- ☐ Vortexer
- ☐ Water bath, thermomixer, or heat block capable of 55°C
- ☐ 100% Ethanol
- ☐ 70% Ethanol
- ☐ Weighing scale
- ☐ Weighing paper
- ☐ Scalpel
- ☐ Dry ice for frozen tissue
- ☐ Liquid Nitrogen
- ☐ Optional: phosphate-buffered saline (PBS) or nuclease-free water may be required

Things to do Before Starting

- ☐ Ensure that HMW1 Buffer and HMW2 Buffer are prepared according to the Preparation of Reagents section on page 2.
- ☐ If performing manual DNA extraction, preset water bath, thermomixer, or heating blocks to 55°C.
- ☐ HMW1 Buffer may show precipitates during storage. If precipitates are present, heat the bottle to 37°C before use.
- ☐ Make sure to resuspend the MAG-HM1 Particles by vortexing before use.

Processing Tips

- ☐ Avoid repeated freeze thaw of samples and store samples properly to avoid DNA shearing.
- ☐ Use low bind tubes to prevent DNA from binding to the tube walls.
- ☐ Use slow, gentle pipetting technique to help keep DNA intact during extraction.
- ☐ To avoid DNA shearing, use wide-bore pipette tips and avoid vortexing.

Protocol

1. Place up to 10 mg of tissue into a 1.5 mL microcentrifuge tube.

   ⚠ *Cutting/mincing the tissue into smaller pieces (≤1 mm^3 pieces) using a scalpel can speed up the lysis process.*

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

2. Add 250 µL of **HTS Buffer** to each sample.

3. Add 20 µL of **Pro K Solution**. Tap the tubes to mix thoroughly or invert mix 5-10 times. Incubate at 55°C in a water bath for 30 minutes (invert 3 times during incubation) or use a thermomixer set at 900 rpm.

4. To remove the liquid from the tube caps, minicentrifuge the tubes for 2 seconds.

5. For tissue samples containing material that cannot be completely digested during the lysis step, centrifuge the plate at 10,000 x g at room temperature (15–30°C) for 5 minutes to pellet the undigested materials.

6. Transfer the clear lysate to a new 1.5 mL tube.

7. Add 5 µL of **RNase A** to each sample tube. Pulse vortex for 1 second and incubate for 3 minutes at room temperature (15–30°C).

8. Add 200 µL of **HAS Buffer** to the sample, invert mix 5-10 times or pulse vortex for 1 sec x 5 times (max setting), and incubate at 55°C for 10 minutes. Gently invert the sample 5-10 times to mix once during the incubation.

   ⚠ *From this step, no vortexing or pipette mixing is allowed during the extraction processing, except during the elution step.*

9. Add 290 µL of 100% Ethanol and 10 µL of **MAG-HM1 Particles** to the sample. Gently invert the tube 2-5 times to mix. Put the sample on a rotator and rotate gently at room temperature for 10 minutes. It is normal for lumps of beads and DNA to form during rotation. Do not break up the lumps. Briefly spin the tube on a minicentrifuge for 2 seconds.

10. Place the sample on a magnetic separation device for 5 minutes or until the **MAG-HM1 Particles** are completely cleared from the solution. Remove and discard all of the liquid.

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

11. Remove the sample from the magnetic separation device. Add 500 µL of **HMW1 Buffer** and put the sample on a rotator. Gently rotate at room temperature for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds.

    ⚠ ***HMW1 Buffer** must be diluted with 100% Ethanol prior to use.*

12. Place the sample on the magnetic separation device for 2 minutes or until the **MAG-HM1 Particles** are completely cleared from the solution. Remove and discard all the liquid.

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

13. Repeat Steps 11-12 for a second **HMW1 Buffer** wash.

14. Remove the sample from the magnetic separation device. Add 500 µL of **HMW2 Buffer** and put the sample on a rotator. Gently rotate at room temperature for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds.

    ⚠ ***HMW2 Buffer** must be diluted with 100% Ethanol prior to use.*

15. Place the sample on a magnetic separation device for 2 minutes or until the **MAG-HM1 Particles** are completely cleared from the solution. Remove and discard all the liquid.

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

16. Repeat Steps 14-15 for a second **HMW2 Buffer** wash.

17. Keep the sample on the magnetic separation device and air dry the magnetic beads at room temperature for 5 minutes. Remove any residual liquid with a pipette.

    ⚠ *It is critical to completely remove all liquid from the tube.*

18. Add 50-100 µL of **MB Elution Buffer** to the sample. Do not use a pipette or vortex to mix the sample.

    ⚠ *Do not pipette mix the beads and elution buffer before heating because the mass of the clumps will shred the DNA fragments.*

19. Incubate the sample at 55°C for 5 minutes and then remove the sample from 55°C incubation. Gently pipette mix the sample 5 times. Then, incubate the sample at 55°C for an additional 5 minutes.

20. Place the sample back on the magnetic separation device for 5 minutes or until the **MAG-HM1 Particles** are completely cleared from the **MB Elution Buffer**.

21. Transfer the eluate (cleared supernatant) to an appropriate storage vessel using a wide-bore P200 pipette. Keep the DNA at 4°C for subsequent applications. For long term storage, keep the DNA at -20°C.

    ⚠ *Do not freeze and thaw HMW DNA repeatedly. This will break the DNA into smaller pieces.*

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	Frozen samples not mixed properly after thawing	Thaw the frozen samples at room temperature and gently mix the samples by inverting
	Blood is too old	Best yields are obtained from fresh blood
	Low levels of leukocytes	Low white blood cells count will give reduced yield
	Incomplete resuspension of MAG-HM1 Particles	Resuspend the MAG-HM1 Particles by vortexing vigorously before use
	Loss of MAG-HM1 Particles during operation	Avoid disturbing the MAG-HM1 Particles during aspiration of supernatant
	DNA remains bound to the MAG-HM1 Particles	Increase elution volume and incubate for 15 minutes. Pipette mix 50 to 100 times
	Ethanol is not added into HMW1 or HMW2 Buffer	Add absolute 100% Ethanol to HMW1 or HMW2 Buffer (see page 2 for instructions)
MAG-HM1 Particles do not completely clear from the solution	Too short of magnetizing time	Increase collection time on the magnet.
Eluted DNA contains gelatinous material	Blood is too old	Remove the gelatinous material by centrifugation. Recommend using fresh blood
		Use 8 mM NaOH as elution buffer
Problems in downstream applications	Insufficient DNA in starting material	Use more starting material
	Ethanol carry-over	Dry the MAG-HM1 Particles completely before elution

Ordering

HighPrep High Molecular Weight DNA Kit

Catalog No.	Product	Description	Preps
HPHMW-D96	HighPrep HMW DNA Kit (96 Preps)	High molecular weight DNA isolation from whole blood, bone marrow, saliva, buccal cells, cultured cells, tissues, and bacteria	96
HPHMW-D96x4	HighPrep HMW DNA Kit (384 Preps)		384

Related Products

HighPrep PCR PB

Catalog No.	Product	Description
PB-60005	HighPrep PCR PB (5 mL)	Cleanup and size selection for long read sequencing
PB-60050	HighPrep PCR PB (50 mL)	

Short Fragment Depletor - 10HT

Catalog No.	Product	Description
SF-80005	Short Fragment Depletor - 10HT (5 mL)	Progressive depletion of DNA fragments <10 kb
SF-80050	Short Fragment Depletor - 10HT (50 mL)	

Magnetic Separation Devices

Catalog No.	Description
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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