Auto-Mag® HMW DNA Isolation Kit

Version 2.2

Magnetic bead-based kit for automated or manual isolation of high molecular weight DNA from whole blood, saliva, buccal cells, cultured cells, tissues, and bacteria, etc.

Catalog Number: D036-00, D036-01, D036-02

Contents

Disclaimers and Safety Information

This kit is designed for research use only. All biological samples are considered potentially infectious. 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 Documents" tab when viewing the product kit. Download MSDS at www.amdbiotech.com. Information in this document is subject to change without notice.

Product Introduction

Auto-Mag® High Molecular Weight (HMW) DNA Isolation utilizes paramagnetic bead-based technology and is designed for Isolation of High-Molecular-Weight DNA in the 20-300+kb size ranges from blood, saliva, issue cultured cells and bacteria samples. The HMW DNA purified with this system is particularly suitable for analysis on long-range genomics platforms including PacBio RSII/Sequel/Sequel II and Oxford Nanopore.

The Auto-Mag® HMW DNA Isolation Kit contains sufficient reagents for the specified number of preparations. The kit also includes RNase A for RNA removal and a special lytic enzyme mix for bacterial HMW DNA Isolation. The DNA purified using this kit and procedure is of good quality and purity. As a reminder, the exact size of extracted DNA varies depending on sample matrix, the quality of the starting material, and processing parameters. High-quality samples are the key to obtaining high-quality HMW DNA. Either fresh or frozen samples can be used equivalently with this kit. However, care should be taken to minimize freeze-thaws. To maximize genomic DNA size, the protocols for this kit do not incorporate pipette mixing or vortex mixing of the sample during the Isolation procedures, except during the elution step.

Additionally, If the extracted HMW DNA contains unwanted small DNA fragments due to sample quality, we recommend cleaning up the DNA by using Auto-Mag® Mega select (AMD-S005) to eliminate small fragments to ensure recovered high-quality HMW DNA meets Long-Read Sequencing requirements.

Features:

- Reproducibly purify high molecular weight genomic DNA (50-300+kb) from a wide range of samples matrices.
- Simplified, user friendly protocols that remove RNA and inhibitors.
- Comes with enzymes mixture for isolation high quality HMW DNA from Bacteria
- HMW DNA is suitable for all third-generation sequencing platforms including Nanopore and PacBio SMRT sequencing.
- Automation-friendly and can be easily scaled up.

Kit Contents

Product Number	**D036-00**	**D036-01**	**D036-02**
Preparation	5	96	384
Auto-Mag® HD-1	0.06 ml	1.1 ml	4.2 ml
HMWL Buffer	3 ml	50 ml	200 ml
HMWB Buffer	1.8 ml	32 ml	125 ml
HMWW Buffer*	4 ml	60 ml	120 ml x2
DNAW Buffer*	2 ml	35 ml	70 ml x 2
DNA Elution Buffer	1.2 ml	20 ml	80 ml
Proteinase K Solution	0.11 ml	2 ml	8 ml
RNase A	0.03 ml	0.55ml	2.2 ml
LEM-2 Mix	0.11 ml	2 ml	8 ml
*** Ethanol must be added prior to use. See Preparation of Reagents**			

Storage and Stability

Auto-Mag® HMW DNA Isolation Kit is shipped at ambient temperature. All components are stable for 12 months when stored accordingly: LEM-2 is stable at 2-8°C (12 months) and for longer storage, keep at -20°C. Auto-Mag® HD-1 beads, Proteinase K Solution and RNase A can be stored at room temperature (15-25°C) for 12 months, to prolong the shelf-life, storage at 2-8°C is recommended. All other components can be stored at room temperature (15-25°C).

During shipment or storage in cool ambient conditions, the precipitates may form in some buffers. Check buffers and re-dissolve any precipitates by warming the buffer at 37˚C. and gently shaking before using.

Preparation of Reagents

1. Dilute HMWW Buffer with 100% Ethanol as follows and store at room temperature.

Reagents	Kit	100% Ethanol to be Added
HMWW Buffer	D036-00	4 ml
	D036-01	60 ml
	D036-02	120 ml per bottle
Components are stable for 1 year when stored closed at room temperature		

2. Dilute DNAW Buffer with 100% Ethanol as follows and store at room temperature.

Reagents	Kit	100% Ethanol to be Added
DNAW Buffer	D036-00	5 ml
	D036-01	85 ml
	D036-02	170 ml per bottle
Components are stable for 1 year when stored closed at room temperature		

Additional Information

1. Specifications

Features	Specification
Isolation Technology	Magnetic bead-based technology
Sample Sources	Blood, saliva, culture cells, Tissue, Bacteria, etc.
Starting Amount	Up to 200 µl of blood or saliva, up to 10mg tissue, $1x10^{6-9}$ cells, or bacterial pellet from 1 ml culture with OD_{600}=1 or less.
DNA Size Range	50-300+kb

Typical Yield	Dependent upon sample, (2-20µg)
A260/280	1.8-2.0
Elution Volume	50-100 µl
Processing format	Manual, Automated
Downstream Application	Long read sequencing, Next generation sequencing, Real-time quantitative PCR, Microarray, Genotyping, Restriction enzyme digestion

2. Amounts of starting material

Sample	Amount
Human whole Blood	~200µl
Saliva	~200µl
Culture Cells	$0.5x10^6$ – $1x10^9$ cells
Most tissue samples	5-10mg
Bacteria	1ml of bacteria culture with OD_{600} 0.8-1.0

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3. The short overview of the HMW DNA isolation.

1. Pre-Processing samples: Homogenization of solid tissues, centrifugation or pelleting of cells from collection or growth medium.
2. Lysis cells and release DNA: Cell lysis is carried out by HMWL buffer, HMWB buffer, Proteinase K and LEM-2 Mix.
3. Remove RNA: RNase A degrades RNA in the sample.
4. DNA Binding: Auto-Mag® HD-1 Particles in the presence of 100% Ethanol and a chaotropic salt bind the DNA in the solution.
5. Bead Wash: Wash of Auto-Mag® HD-1 Particles with HMWW Wash Buffer and DNAW Wash Buffer
6. DNA Elution: Use a low molarity Buffer to releases DNA from the beads.

Auto-Mag® HMW DNA Isolation Protocols

Materials and Equipment to Be Supplied by User

- Single-tube format: Nuclease-free 1.7-2.0 ml microcentrifuge tube,
- Magnetic Rack Separator for 1.5 ml microcentrifuge tube
- Wide-bore pipette tips (1,000µl and 200µl)
- Mini-Tube Rotator
- Centrifuge
- Thermomixer
- Water bath, incubator, or heat block capable of 55°C and 70°C
- 100% Ethanol
- 70% Ethanol
- 1X Phosphate-buffered saline (PBS) or nuclease-free water may be required

Before Starting

- Equilibrate samples to room temperature.
- Ensure HMWW Buffer and DNAW Buffer are prepared according to the instructions of Preparation Reagents on page 3.
- Preset water bath, incubator, or heating blocks to 55°C.
- HMWB Buffer may show precipitates during storage. If precipitates are present, heat the bottle to 37°C before use.
- Complete resuspension of the Auto-Mag® HD-1 Beads by vortex.

Processing Tips

Pipetting

When adding or removing liquid from the microcentrifuge tube, do not disturb beads. Carefully insert the pipette tip against the wall to dispense, Likewise, remove liquid by pipetting from the liquid surface. This will minimize the chances of accidentally pipetting bound DNA.

Protocol for Isolating HMW DNA from Whole Blood or Saliva Samples

1. Pre-treat the sample:

   1) For whole blood samples

   Gently rock the tube of whole blood samples until thoroughly mixed. Transfer 200µl of whole blood sample to a Nuclease-free 1.7-2.0 ml microcentrifuge tube with a standard P200 pipette.

   Note: Blood must be collected in EDTA, heparin or citrate anticoagulant tubes to prevent clotting.

   2) For saliva samples

   a). The freshly collected saliva samples: Gently rock the tube of samples, Transfer 200µl of sample to a Nuclease-free 1.7-2.0 ml microcentrifuge tube with a standard P200 pipette.

   b). If saliva samples are from preserved or stabilized, Verify with manufacturer recommendations of saliva collection devices if samples require an upfront incubation before sample processing. and then, Transfer 200µl of sample to a Nuclease-free 1.7-2.0 ml microcentrifuge tube.

2. Add 100µl of HMWL Buffer, and 20µl of Proteinase K to the sample. Pipette mix 10 times with a standard P200 pipette to make sure there are no visible clotting, and the sample looks homogenous.

3. Add 300µl of HMWB Buffer to the sample. Pulse vortex for 1 second (max setting) or invert the tube 5–10 times to mix.

4. Incubate the samples at 55°C in a thermomixer (300 rpm) or heat block or water bath (needs periodic agitation-invert the tube at least 3-5 times during incubation) for 20 minutes.

Note: From this step on, no vortex or pipette mix is allowed during the Isolation processing, except elution step.

5. Bring the sample to room temperature. Add 5µl of RNase A. Invert the tube 5–10 times and incubate for 3 minutes.

6. Using wide bore pipette tips, add 400µl of 100 % Ethanol and 10µl of Auto-Mag® HD-1 to the sample. Gently invert the tube 2-5 times to mix.

   Note: 100% Ethanol and Auto-Mag® HD-1 can be prepared as a master mix prior to use. Prepare only what is needed and mix completely. Complete resuspension of the Auto-Mag® HD-1 beads are crucial for obtaining purity

7. Place the sample on the Mini-Tube Rotator and rotate it at room temperature for 10 minutes. It is normal for lumps of beads and DNA to form during rotation, and do not try to break up them.

   Note: If there are some lumps attached to the tube cover, briefly spin the tube on a minicentrifuge for 2 seconds.

8. Place the sample on a compatible magnetic separation device for 5 minutes or until Auto-Mag® HD-1 beads are completely cleared from solution. Discard the supernatant by pipetting. Do not disturb the attracted beads.

9. Remove the sample from the magnet. Using wide bore pipette tips, gently add 600µl of HMWW Buffer and put

sample on the Mini-Tube Rotator for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds if necessary.

Note: HMWW Buffer must be diluted with ethanol prior to use.

10. Place the sample on the magnet for 2 minutes or until Auto-Mag® HD-1 beads are completely cleared from solution. Discard the supernatant by pipetting. Do not disturb the attracted beads.

11. Repeat Steps 9-10 for a second HMWW Buffer wash.

12. Remove the sample from the magnet. Using wide bore pipette tips, gently add 600µl of DNAW Buffer and put sample on the Mini-Tube Rotator for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds if necessary.

Note: DNAW Buffer must be diluted with ethanol prior to use.

13. Place the sample on the magnet for 2 minutes or until Auto-Mag® HD-1 beads are completely cleared from solution. Discard the supernatant by pipetting. Do not disturb the attracted beads.

14. Repeat Steps 12-13 for a second DNAW Buffer wash.

15. Keep the sample on the magnet, and air dry the magnetic beads at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

Note: It is critical to completely remove all liquid from each well.

16. Add 50~150µl Elution Buffer to the sample and ensure that the beads are submerged in the elution buffer. **Do not pipette or vortex to mix the sample**, first incubate the sample at 55°C for 5 minutes.

Note: Please don't pipet mix beads and elution buffer before heating because the mass of the clumps will shred the DNA fragments.

17. Remove the sample from 55°C incubation, gently mix the sample by pipetting 10 times, and then put sample back to 55°C and incubate sample again for additional 5 minutes.

18. Place the sample back on the magnet for 5 minutes or until the Auto-Mag® HD-1 beads are completely cleared from elution buffer.

Note: If there are gel like consistency during the elution step, and the beads are not completely clear after 20 minutes of magnetization: please proceed to step 20.

19. Transfer the eluate (cleared supernatant) to an appropriate storage vessel and keep at 4°C or for subsequent applications. For long term storage, keep DNA at -20°C.

Note: Do not freeze and thaw HMW DNA repeatedly, it will disrupt the DNA.

20. Additional, a small amount of gel like material may remain adhesion on the beads after the eluate incubation in step 18. This clear gel is DNA. Let sample rest at room temperature overnight to allow DNA to solubilize. Visible "jellies" should disperse after resting. Following overnight rest, pipette mix 10 times with a standard P200 pipette, then repeat step 17-19 to elute the DNA.

Protocol for Isolating HMW DNA from Buccal Swab Sample

1. Pre-treat the sample:

   For buccal swab sample: Transfer buccal swab sample to a sterile microcentrifuge tube. Add 0.5 ml of HMWL Buffer to the tube, making sure that the sample is completely immersed in the HMWL Buffer. Incubate the sample at 37°C for 20 minutes. Transfer ~300µl pre-treated sample to a Nuclease-free 1.7-2.0 ml microcentrifuge tube,

2. Transfer ~300µl pre-treated sample to a Nuclease-free 1.7-2.0 ml microcentrifuge tube. Add 20µl of Proteinase K, to the sample. Pipette mix 10 times with a standard P200 pipette to make sure there are no visible clotting, and the sample looks homogenous.

3. Add 300µl of HMWB Buffer to the sample. Pulse vortex for 1 second (max setting) or invert the tube 5–10 times to mix.

4. Incubate the samples at 55°C in a thermomixer (300 rpm) or heat block or water bath (needs periodic agitation-invert the tube at least 3-5 times during incubation) for 20 minutes.

Note: From this step on, no vortex or pipette mix is allowed during the Isolation processing, except elution step.

5. Bring the sample to room temperature. Add 5µl of RNase A. Invert the tube 5–10 times and incubate for 3 minutes.

6. Using wide bore pipette tips, add 400µl of 100 % Ethanol and 10µl of Auto-Mag® HD-1 to the sample. Gently invert the tube 2-5 times to mix.

   Note: 100% Ethanol and Auto-Mag® HD-1 can be prepared as a master mix prior to use. Prepare only what is needed and mix completely. Complete resuspension of the Auto-Mag® HD-1 beads are crucial for obtaining purity

7. Place the sample on the Mini-Tube Rotator and rotate it at room temperature for 10 minutes. It is normal for lumps of beads and DNA to form during rotation, and do not try to break up them.

   Note: If there are some lumps attached to the tube cover, briefly spin the tube on a minicentrifuge for 2 seconds.

8. Place the sample on a compatible magnetic separation device for 5 minutes or until Auto-Mag® HD-1 beads are completely cleared from solution. Discard the supernatant by pipetting. Do not disturb the attracted beads.

9. Remove the sample from the magnet. Using wide bore pipette tips, gently add 600µl of HMWW Buffer and put sample on the Mini-Tube Rotator for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds if necessary.

   Note: HMWW Buffer must be diluted with ethanol prior to use.

10. Place the sample on the magnet for 2 minutes or until Auto-Mag® HD-1 beads are completely cleared from solution. Discard the supernatant by pipetting. Do not disturb the attracted beads.

11. Repeat Steps 9-10 for a second HMWW Buffer wash.

12. Remove the sample from the magnet. Using wide bore pipette tips, gently add 600µl of DNAW Buffer and put sample on the Mini-Tube Rotator for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds if necessary.

Note: DNAW Buffer must be diluted with ethanol prior to use.

13. Place the sample on the magnet for 2 minutes or until Auto-Mag® HD-1 beads are completely cleared from solution. Discard the supernatant by pipetting. Do not disturb the attracted beads.

14. Repeat Steps 12-13 for a second DNAW Buffer wash.

15. Keep the sample on the magnet, and air dry the magnetic beads at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

Note: It is critical to completely remove all liquid from each well.

16. Add 50~150µl Elution Buffer to the sample and ensure that the beads are submerged in the elution buffer. **Do not pipette or vortex to mix the sample**, first incubate the sample at 55°C for 5 minutes.

Note: Please don't pipet mix beads and elution buffer before heating because the mass of the clumps will shred the DNA fragments.

17. Remove the sample from 55°C incubation, gently mix the sample by pipetting 10 times, and then put sample back to 55°C and incubate sample again for additional 5 minutes.

18. Place the sample back on the magnet for 5 minutes or until the Auto-Mag® HD-1 beads are completely cleared from elution buffer.

Note: If there are gel like consistency during the elution step, and the beads are not completely clear after 20 minutes of magnetization: please proceed to step 20.

19. Transfer the eluate (cleared supernatant) to an appropriate storage vessel and keep at 4°C or for subsequent applications. For long term storage, keep DNA at -20°C.

Note: Do not freeze and thaw HMW DNA repeatedly, it will disrupt the DNA.

20. Additional, a small amount of gel like material may remain adhesion on the beads after the eluate incubation in step 18. This clear gel is DNA. Let sample rest at room temperature overnight to allow DNA to solubilize. Visible "jellies" should disperse after resting. Following overnight rest, pipette mix 10 times with a standard P200 pipette, then repeat step 17-19 to elute the DNA.

Protocol for Isolating HMW DNA from Cell Culture Sample

1. Prepare the cultured cell suspension according to your starting sample method:

   a. Frozen cell samples should be thawed before starting this protocol. Pellet cells by centrifugation at 1,200 x g for 5 minutes. Add 500µl of cold PBS (4°C), gently invert the tube 2-5 times to resuspend cells. Pellet cells by centrifugation at 1,200 x g for 5 minutes. discard the supernatant.

   b. For cells grown in suspension, pellet $5x10^6$ cells by centrifuge at 1,200 x g for 5 minutes. Discard the supernatant. Add 500µl of cold PBS (4°C), gently invert the tube 2-5 times to resuspend cells. Pellet cells by centrifugation at 1,200 x g for 5 minutes. discard the supernatant.

   c. For cells grown in a monolayer, harvest the cells by either using a trypsin treatment or cell scraper. Pellet cells by centrifugation at 1,200 x g for 5 minutes. Add 500µl of cold PBS (4°C), gently invert the tube 2-5 times to resuspend cells. Pellet cells by centrifugation at 1,200 x g for 5 minutes. discard the supernatant.

2. Add 300µl of HMWL Buffer, and 20µl of Proteinase K to the sample. Pipette mix 10 times with a standard P200 pipette to make sure there are no visible clotting, and the sample looks homogenous.

3. Add 300µl of HMWB Buffer to the sample. Pulse vortex for 1 second (max setting) or invert the tube 5–10 times to mix.

4. Incubate the samples at 55°C in a thermomixer (300 rpm) or heat block or water bath (needs periodic agitation-invert the tube at least 3-5 times during incubation) for 20 minutes.

Note: From this step on, no vortex or pipette mix is allowed during the Isolation processing, except elution step.

5. Bring the sample to room temperature. Add 5µl of RNase A. Invert the tube 5–10 times and incubate for 3 minutes.

6. Using wide bore pipette tips, add 400µl of 100 % Ethanol and 10µl of Auto-Mag® HD-1 to the sample. Gently invert the tube 2-5 times to mix.

   Note: 100% Ethanol and Auto-Mag® HD-1 can be prepared as a master mix prior to use. Prepare only what is needed and mix completely. Complete resuspension of the Auto-Mag® HD-1 beads are crucial for obtaining purity

7. Place the sample on the Mini-Tube Rotator and rotate it at room temperature for 10 minutes. It is normal for lumps of beads and DNA to form during rotation, and do not try to break up them.

   Note: If there are some lumps attached to the tube cover, briefly spin the tube on a minicentrifuge for 2 seconds.

8. Place the sample on a compatible magnetic separation device for 5 minutes or until Auto-Mag® HD-1 beads are completely cleared from solution. Discard the supernatant by pipetting. Do not disturb the attracted beads.

9. Remove the sample from the magnet. Using wide bore pipette tips, gently add 600µl of HMWW Buffer and put sample on the Mini-Tube Rotator for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds if necessary.

   Note: HMWW Buffer must be diluted with ethanol prior to use.

10. Place the sample on the magnet for 2 minutes or until Auto-Mag® HD-1 beads are completely cleared from solution. Discard the supernatant by pipetting. Do not disturb the attracted beads.

11. Repeat Steps 9-10 for a second HMWW Buffer wash.

12. Remove the sample from the magnet. Using wide bore pipette tips, gently add 600µl of DNAW Buffer and put sample on the Mini-Tube Rotator for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds if necessary.

Note: DNAW Buffer must be diluted with ethanol prior to use.

13. Place the sample on the magnet for 2 minutes or until Auto-Mag® HD-1 beads are completely cleared from solution. Discard the supernatant by pipetting. Do not disturb the attracted beads.

14. Repeat Steps 12-13 for a second DNAW Buffer wash.

15. Keep the sample on the magnet, and air dry the magnetic beads at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

Note: It is critical to completely remove all liquid from each well.

16. Add 50~150µl Elution Buffer to the sample and ensure that the beads are submerged in the elution buffer. **Do not pipette or vortex to mix the sample**, first incubate the sample at 55°C for 5 minutes.

Note: Please don't pipet mix beads and elution buffer before heating because the mass of the clumps will shred the DNA fragments.

17. Remove the sample from 55°C incubation, gently mix the sample by pipetting 10 times, and then put sample back to 55°C and incubate sample again for additional 5 minutes.

18. Place the sample back on the magnet for 5 minutes or until the Auto-Mag® HD-1 beads are completely cleared from elution buffer.

Note: If there are gel like consistency during the elution step, and the beads are not completely clear after 20 minutes of magnetization: please proceed to step 20.

19. Transfer the eluate (cleared supernatant) to an appropriate storage vessel and keep at 4°C or for subsequent applications. For long term storage, keep DNA at -20°C.

Note: Do not freeze and thaw HMW DNA repeatedly, it will disrupt the DNA.

20. Additional, a small amount of gel like material may remain adhesion on the beads after the eluate incubation in step 18. This clear gel is DNA. Let sample rest at room temperature overnight to allow DNA to solubilize. Visible "jellies" should disperse after resting. Following overnight rest, pipette mix 10 times with a standard P200 pipette, then repeat step 17-19 to elute the DNA.

Protocol for Isolating HMW DNA from Tissue Sample

1. Place up to 5-10mg tissue into a 1.5-2.0 ml microcentrifuge tube.

   Note: Cutting/mincing the tissue into smaller pieces can speed up the lysis process

2. Add 300µl of HMWL Buffer and 20µl Proteinase K Solution to each sample and gently invert the tube 2-5 times.

3. Incubate the sample at 55°C in a shaking water bath overnight. Overnight lysis is recommended for optimal yield.

   Note: Alternatively, lysis can be performed in 2-4 hours depending on the amount and tissue type. If a shaking water bath is not available, gently invert the tube 2-5 times every 20-30 minutes.

   For tissues samples containing material that cannot be digested during the lysis step, centrifuge the sample at 1,200 x g for 5 minutes to pellet the undigested materials. Transfer all the clear lysate to a new tube.

4. Add 5µl RNase A to the sample and gently invert the tube 2-5 times. Incubate at room temperature for 3 minutes.

   Note: from this step, no vortex or pipette mix is allowed during the Isolation processing, except Elution step.

5. Using wide bore pipette tips, add 300µl of HMWB Buffer and gently invert the tube 2-5 times to mix.

6. Using wide bore pipette tips, add 400µl of 100 % Ethanol and 10µl of Auto-Mag® HD-1 to the sample. Gently invert the tube 2-5 times to mix.

   Note: 100% Ethanol and Auto-Mag® HD-1 can be prepared as a master mix prior to use. Prepare only what is needed and mix completely. Complete resuspension of the Auto-Mag® HD-1 beads are crucial for obtaining purity

7. Place the sample on the Mini-Tube Rotator and rotate it at room temperature for 10 minutes. It is normal for lumps of beads and DNA to form during rotation, and do not try to break up them.

   Note: If there are some lumps attached to the tube cover, briefly spin the tube on a minicentrifuge for 2 seconds.

8. Place the sample on a compatible magnetic separation device for 5 minutes or until Auto-Mag® HD-1 beads are completely cleared from solution. Discard the supernatant by pipetting. Do not disturb the attracted beads.

9. Remove the sample from the magnet. Using wide bore pipette tips, gently add 600µl of HMWW Buffer and put sample on the Mini-Tube Rotator for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds if necessary.

   Note: HMWW Buffer must be diluted with ethanol prior to use.

10. Place the sample on the magnet for 2 minutes or until Auto-Mag® HD-1 beads are completely cleared from solution. Discard the supernatant by pipetting. Do not disturb the attracted beads.

11. Repeat Steps 9-10 for a second HMWW Buffer wash.

12. Remove the sample from the magnet. Using wide bore pipette tips, gently add 600µl of DNAW Buffer and put sample on the Mini-Tube Rotator for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds if necessary.

Note: DNAW Buffer must be diluted with ethanol prior to use.

13. Place the sample on the magnet for 2 minutes or until Auto-Mag® HD-1 beads are completely cleared from solution. Discard the supernatant by pipetting. Do not disturb the attracted beads.

14. Repeat Steps 12-13 for a second DNAW Buffer wash.

15. Keep the sample on the magnet, and air dry the magnetic beads at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

Note: It is critical to completely remove all liquid from each well.

16. Add 50~150µl Elution Buffer to the sample and ensure that the beads are submerged in the elution buffer. **Do not pipette or vortex to mix the sample**, first incubate the sample at 55°C for 5 minutes.

Note: Please don't pipet mix beads and elution buffer before heating because the mass of the clumps will shred the DNA fragments.

17. Remove the sample from 55°C incubation, gently mix the sample by pipetting 10 times, and then put sample back to 55°C and incubate sample again for additional 5 minutes.

18. Place the sample back on the magnet for 5 minutes or until the Auto-Mag® HD-1 beads are completely cleared from elution buffer.

Note: If there are gel like consistency during the elution step, and the beads are not completely clear after 20 minutes of magnetization: please proceed to step 20.

19. Transfer the eluate (cleared supernatant) to an appropriate storage vessel and keep at 4°C or for subsequent applications. For long term storage, keep DNA at -20°C.

Note: Do not freeze and thaw HMW DNA repeatedly, it will disrupt the DNA.

20. Additional, a small amount of gel like material may remain adhesion on the beads after the eluate incubation in step 18. This clear gel is DNA. Let sample rest at room temperature overnight to allow DNA to solubilize. Visible "jellies" should disperse after resting. Following overnight rest, pipette mix 10 times with a standard P200 pipette, then repeat step 17-19 to elute the DNA.

Protocol for Isolating HMW DNA from cultured Gram-Negative/Positice Bacteria

This protocol is suitable for Isolation HMW bacteria DNA, including plasmid DNA from a wide variety of bacterial species.

Bacteria cell sample input requirements: 1ml of bacteria culture with OD_{600} 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 Isolation reagents.

1. Harvest bacterial cells by centrifuging the culture at 16,000 x g for 1 minute at room temperature. Discard the culture supernatant. The pellet can be processed as a fresh pellet or stored frozen for late processing.

2. Add 200µl of 1x PBS. and 20µl of LEM-2 to the sample. Pipette mix 10 times with a standard P200 pipette to resuspend the bacterial pellet. Make sure there are no visible cell lumps and incubate samples at room temperature for 30 minutes.

3. Add 20µl of Proteinase K and 200µl of HMWB Buffer to the sample. Pulse vortex for 1 second (max setting) or invert the tube 5–10 times to mix. Incubate the samples at 55°C in a thermomixer (300 rpm) or heat block or water bath (needs periodic agitation-invert the tube at least 3-5 times during incubation) for 20 minutes.

   Note: from this step, no vortex or pipette mix is allowed during the Isolation processing, except Elution step.

4. Bring the sample to room temperature. Add 5µl of RNase A. Invert the tube 5–10 times and incubate for 3 minutes.

5. Incubate the samples at 70°C in a thermomixer (300 rpm) or heat block or water bath (needs periodic agitation-invert the tube at least 3-5 times during incubation) for 20 minutes.

6. Using wide bore pipette tips, add 300µl of 100 % Ethanol and 10µl of Auto-Mag® HD-1 to the sample. Gently invert the tube 2-5 times to mix.

   Note: 100% Ethanol and Auto-Mag® HD-1 can be prepared as a master mix prior to use. Prepare only what is needed and mix completely. Complete resuspension of the Auto-Mag® HD-1 beads are crucial for obtaining purity

7. Place the sample on the Mini-Tube Rotator and rotate it at room temperature for 10 minutes. It is normal for lumps of beads and DNA to form during rotation, and do not try to break up them.

   Note: If there are some lumps attached to the tube cover, briefly spin the tube on a minicentrifuge for 2 seconds.

8. Place the sample on a compatible magnetic separation device for 5 minutes or until Auto-Mag® HD-1 beads are completely cleared from solution. Discard the supernatant by pipetting. Do not disturb the attracted beads.

9. Remove the sample from the magnet. Using wide bore pipette tips, gently add 600µl of HMWW Buffer and put sample on the Mini-Tube Rotator for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds if necessary.

   Note: HMWW Buffer must be diluted with ethanol prior to use.

10. Place the sample on the magnet for 2 minutes or until Auto-Mag® HD-1 beads are completely cleared from solution. Discard the supernatant by pipetting. Do not disturb the attracted beads.

11. Repeat Steps 9-10 for a second HMWW Buffer wash.

12. Remove the sample from the magnet. Using wide bore pipette tips, gently add 600µl of DNAW Buffer and put sample on the Mini-Tube Rotator for 5 minutes. Briefly spin the tube on a minicentrifuge for 2 seconds if necessary.

    Note: DNAW Buffer must be diluted with ethanol prior to use.

13. Place the sample on the magnet for 2 minutes or until Auto-Mag® HD-1 beads are completely cleared from solution. Discard the supernatant by pipetting. Do not disturb the attracted beads.

14. Repeat Steps 12-13 for a second DNAW Buffer wash.

15. Keep the sample on the magnet, and air dry the magnetic beads at room temperature for 5 minutes. Remove any residue liquid with a pipettor.

    Note: It is critical to completely remove all liquid from each well.

16. Add 50~150µl Elution Buffer to the sample and ensure that the beads are submerged in the elution buffer. **Do not pipette or vortex to mix the sample**, first incubate the sample at 55°C for 5 minutes.

    Note: Please don't pipet mix beads and elution buffer before heating because the mass of the clumps will shred the DNA fragments.

17. Remove the sample from 55°C incubation, gently mix the sample by pipetting 10 times, and then put sample back to 55°C and incubate sample again for additional 5 minutes.

18. Place the sample back on the magnet for 5 minutes or until the Auto-Mag® HD-1 beads are completely cleared from elution buffer.

    Note: If there are gel like consistency during the elution step, and the beads are not completely clear after 20 minutes of magnetization: please proceed to step 20.

19. Transfer the eluate (cleared supernatant) to an appropriate storage vessel and keep at 4°C or for subsequent applications. For long term storage, keep DNA at -20°C.

    Note: Do not freeze and thaw HMW DNA repeatedly, it will disrupt the DNA.

20. Additional, a small amount of gel like material may remain adhesion on the beads after the eluate incubation in step 18. This clear gel is DNA. Let sample rest at room temperature overnight to allow DNA to solubilize. Visible "jellies" should disperse after resting. Following overnight rest, pipette mix 10 times with a standard P200 pipette, then repeat step 17-19 to elute the DNA.

Protocol and Programmed Procedure for Automated Isolation Process

If automating these procedures on a liquid handler or a magnetic processor, please contact AMD Biotech for instrument-specific instructions and additional processing procedures.

Phone: 404-290-5063 (in US), Email: support@amdbiotech.com

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via: Phone: 404-290-5063 (in US), Email: support@amdbiotech.com

Symptoms	Possible Causes	Comments
	Sample quality does not meet requirements	Use good quality samples. such as fresh blood and saliva,
Low DNA yields	Incomplete resuspension of Auto-Mag® HD-1 beads	Resuspend Auto-Mag® HD-1 beads by vortexing vigorously before use.
	Loss of Auto-Mag® HD-1beads during operation	Avoid disturbing the Auto-Mag® beads during aspiration of supernatant.
	DNA remains bound to the Auto-Mag® HD-1 beads	Increase elution volume and incubate at 65°C for 10 minutes
	Ethanol is not added into HMWW Buffer or DNAW Buffer	Add absolute 100% Ethanol to HMWW Buffer or DNAW Buffer (see Page 2 for instructions).
Auto-Mag® beads do not clear from solution	Too short of magnetizing time	Increase collection time on the magnet
Downstream applications are unsuccessful	Insufficient DNA in starting material	Use more starting material
	Ethanol carry-over	Dry the Auto-Mag® HD-1 beads completely before elution.

Ordering Information

Product Description	Catalog No.	Size
Auto-Mag® HMW DNA Isolation Kit	D036-00 D036-01 D036-02 D036-Bulk	5 Preps 96 Preps 384 Preps Request
Auto-Mag® Mega Select	S005-01 S005-02 S005-03 S005-04	25 Preps 100 Preps 250 Preps 1000 Preps

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If you are dissatisfied with this product for any reason, please contact us.

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