AMD Biotech
ADVANCED MOLECULAR DETECTION

Auto-Mag® HMW DNA Isolation kit

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Auto-Mag® HMW Size Select

An advanced High Molecular Weight (HMW) DNA extraction system; novel and practical HMW DNA fragment size selection reagents and implementation strategies.

Guide & Overview

------2026------

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High Molecular Weight DNA Challenges & Solution

Background Information

High molecular weight (HMW) DNA (50 kb or larger) is essential for studying large-scale genomic variations and applying long-read next-generation sequencing. It enables detailed genome assembly, which is vital in fields like disease research, biodiversity conservation, and structural variation studies. With the advancements in long-read sequencing, achieving high-quality HMW DNA has become crucial for obtaining accurate and comprehensive genomic data.

Current Challenges

Extracting and purifying HMW DNA is challenging, as traditional methods often result in fragmented DNA, impacting long-read sequencing quality. Additionally, degraded sample material or improper extraction techniques can reduce HMW DNA integrity. Existing purification technologies struggle to effectively separate short fragments (<5kb) from HMW DNA, resulting in lower sequencing accuracy on third-generation platforms.

Our Solution

To address the limitations of HMW DNA extraction and purification, AMD Biotech offers new solutions: the **Auto-Mag® HMW DNA Isolation Kit** and the **Auto-Mag® HMW Size Select Kit**. These kits provide gentle and precise HMW DNA isolation and selective removal of small fragments, ensuring only high-quality, high-molecular-weight DNA is retained for sequencing.

Our Approach

Our **Auto-Mag® HMW DNA Isolation Kit** uses nanomagnetic bead technology to avoid DNA fragmentation by bypassing spin columns and high-speed centrifugation, maximizing yield and integrity. For the removal of short fragments of DNA confounded in HMW DNA samples, **Auto-Mag® HMW Size Select** is a new and truly practical magnetic bead-based reagent for short DNA fragment depletion and HMW DNA size selection and cleanup. It allows customizable size selection to eliminate DNA fragments <5kb, <10kb, or <20kb in length from high molecular weight DNA samples to trace levels and recover larger DNA fragments of the desired size.

Auto-Mag® HMW Size Select is suitable for unsheared HMW DNA cleanup and can also be used to selectively remove small DNA fragments while recovering larger DNA fragments from fragmented gDNA or partially degraded gDNA samples. Improved the quality of HMW DNA samples and met the requirements of the third-generation sequencing platform for HMW DNA samples. Dramatically improve average read length on third-generation sequencing platforms such as PacBio SMRT Technology or Oxford Nanopore Technology.

Together, these kits provide a simplified, reliable solution for obtaining high-quality HMW DNA suitable for third generation sequencing platforms, resulting in increased average read length and accuracy.

Products and Package Size

Auto-Mag® HMW DNA Isolation Kit	**D036-00**	**D036-01**	**D036-02**
	Sample	96 preps	384 Preps

Auto-Mag® HMW Size Select	**S005-00**	**S005-01**	**S005-02**	**S005-03**
	Sample	10 ml	50 ml	100 ml

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HMW DNA Isolation

Auto-Mag® HMW DNA Isolation

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

Introduction

Auto-Mag® HMW DNA Isolation Kit is a magnetic bead-based kit and specifically designed for isolation of high molecular weight DNA (HMW DNA) in the size range of 50–300+ kb. The Kit contains sufficient reagents, including RNase A and a special lytic enzyme mix, for HMW DNA extraction from biological samples such as whole blood, bone marrow, saliva, buccal cells, cultured cells, tissues, and bacteria samples.

The Auto-Mag® HMW DNA Isolation Kit utilizes special magnetic beads in combination with chaotropic agents to gently isolate HMW DNA and efficiently remove contaminants and PCR inhibitors while minimizing shearing, resulting in high quality high-molecular-weight genomic DNA. The purified HMW DNA is eluted in a low ionic strength buffer and particularly suitable for analysis on long read sequencing genomic platforms including PacBio RSII/Sequel/Sequel II and Oxford Nanopore.

Auto-Mag® HMW DNA Isolation Kit uses magnetic technology and requires no centrifugation or filtration, it is suitable for manual processing as well as adapting to automatic liquid handling workstations.

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
Wash Buffer 1*	4 ml	60 ml	120 ml x 2
Wash Buffer 2*	2 ml	30 ml	60 ml x 2
DNA Elution Buffer	1.2 ml	20 ml	80 ml
Proteinase K Solution	0.12 ml	2 ml	8 ml
RNase A	0.04 ml	0.55 ml	2.2 ml
LEM-2	0.12 ml	2 ml	8 ml
** Ethanol must be added prior to use. See Preparation of Reagents*			

Preparation of Reagents

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

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

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

Reagents	Kit	100% Ethanol to be Added
Wash Buffer 2	D036-00	Follow bottle instruction
	D036-01	90 ml
	D036-02	180 ml per bottle
Components are stable for 1 year when stored closed at room temperature		

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Sample Information and Pre-Prepare

The size of the extracted HMW DNA can depend on the sample matrix, the quality of the starting material, and the processing parameters. High-quality samples are critical to obtaining high-quality HMW DNA. This kit can be used with fresh or frozen samples, but care should be taken to minimize freeze-thaw cycles.

Blood Samples:

Blood samples must be collected in EDTA, heparin or citrate anticoagulant tubes to prevent clotting. Blood samples can also be flash frozen in liquid nitrogen and stored at -80°C.

Saliva Samples:

a. The freshly collected saliva samples: Gently rock the tube of samples, Transfer 200µl of samples to a Nuclease-free 1.5 ml microcentrifuge tube with a standard P200 pipette for processing DNA isolation.

b. The preserved or stabilized saliva samples: Verify with manufacturer recommendations of saliva collection devices if samples require an upfront treatment before DNA isolation.

Cell Culture Samples:

1-10 million cells or more are ideal for HMW DNA extraction.

a. Frozen cell: The samples should be thawed before starting DNA isolation. 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.

Buccal Swab Samples:

Collect the sample using a standard buccal swab and follow the buccal swab collection instructions. Store it under the required conditions until DNA isolation.

Tissue Samples:

Fresh tissue must be frozen in liquid nitrogen right after harvesting and stored at -80°C. Any freezing thaw cycles should be avoided. Cutting/mincing the tissue into smaller pieces can speed up the lysis process.

Cultured Gram-Negative/Positive Bacteria Samples:

Bacteria cell sample input requirements: Use 1 mL of bacterial culture with an OD_{600} of approximately 1.0. Excessive bacterial cell input may result in incomplete lysis and reduced DNA purity. For cultures with an OD_{600} greater than 1.0, the volumes of the isolation reagents may be increased proportionally.

If the extracted HMW DNA contains plasmid DNA that may interfere with downstream applications, Auto-Mag® HMW Size Select can be used to remove low-molecular-weight plasmid DNA while enriching for high-molecular-weight genomic DNA.

In addition, if you encounter low-quality samples or partially degraded gDNA, it is recommended to further purify the gDNA using our unique Auto-Mag® HMW Size Select reagent (Cat. No. S005) to remove small DNA fragments from the DNA sample and recover HMW DNA of the desired size to meet the needs of long-read sequencing genomic platforms.

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Processing Tips

Pipetting

To maximize the integrity of the extracted genomic DNA, avoid excessive mixing samples with pipettes during the extraction process, except for the elution step. When adding, mixing sample, use wide bore pipette tips. Do not disturb beads when adding or removing liquid from the microcentrifuge tube or well of plate. Carefully insert the pipette tip against the wall and on opposite side towards the beads to dispense or remove liquid.

Wide bore tips

If there are no wide bore tips to mix. Do not vortex, cut the regular tips to resemble wide bore.

Heterogeneity and viscosity

HMW DNA can sometimes be difficult to re-solubilize after extraction and a small amount of gel-like material may remain adhesion on the beads after the eluate incubation. 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, mix 5 times with a standard P200 pipette, then repeat the elution step to elute the DNA.

HMW DNA Isolation Protocols (Single tube format)

Different types of samples may require different lysis steps and conditions. The first 5 steps of the following step-by-step protocol are set according to sample types. Please select the lysis step corresponding to the sample to start DNA extraction. From step 6 to DNA elution step, it is suitable for a variety of samples.

	Sample Type				
	Blood & Saliva	**Buccal Swab**	**Cell Culture**	**Bacteria**	**Tissues**
1.	Transfer 200µl of Blood or Saliva sample to a 1.5 ml microcentrifuge tube.	Transfer buccal swab sample to a 1.5 ml tube. Add 0.5 ml of HMWL Buffer, 5µl of RNase A, 20µl of Proteinase K to the tube, making sure that the sample is completely immersed in the HMWL Buffer.	Pre-prepare the cultured cell pellet as described in " Sample Information and Pre-Prepare ".	Harvest bacterial cells by centrifuging the culture at 16,000 x g for 1 minute at room temperature. Discard the culture supernatant.	Place up to 5-10mg tissue into a 1.5 ml microcentrifuge tube.
2.	Add 100µl of HMWL Buffer, 20µl of Proteinase K, and 5µl of RNase A to the sample. Pulse vortex for 1 second (max setting) or invert the tube 5 times to mix thoroughly.	Incubate the sample at 37°C for 20 minutes. During incubation, gently agitate the tube several times to ensure thorough mixing	Add 300 µl of HMWL Buffer, 20 µl of Proteinase K, and 5 µl RNase A to the sample Pulse vortex for 1 second (max setting) or invert the tube 5 times to mix thoroughly.	Add 300 µL of 1× PBS, 20 µL of LEM-2, and 20 µL of Proteinase K to the sample. Thoroughly resuspend the pellet by pipetting up and down 20 times or vortexing for 20 seconds	Add 300 µl of HMWL Buffer and 20 µl of Proteinase K Solution to sample and gently invert the tube 2-5 times.
3.	Add 300µl of HMWB Buffer to the sample. Pulse vortex for 1 second (max setting) or invert the tube 5 times to mix thoroughly.	Transfer 300µl of the lysate to a new microcentrifuge tube. Add 300µl of HMWB Buffer and pulse vortex for 1 second (max setting) or invert the tube 5 times to mix thoroughly.	Add 300 µl of HMWB Buffer to the sample. Pulse vortex for 1 second (max setting) or invert the tube 5 times to mix thoroughly.	Incubate the samples at room temperature for 10 minutes, then transfer them to 55°C and incubate for an additional 20 minutes. Periodically agitate the samples by gently inverting the tubes 3–5 times.	Incubate the sample in a shaking water bath at 55°C for 2–4 hours. If necessary, the incubation may be extended overnight to achieve optimal lysis and DNA yield.

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4.	Incubate the samples at 55°C for 15 minutes. During incubation, gently invert the tubes 3–5 times to ensure thorough mixing.	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 10 minutes.	Incubate the samples at 55°C for 15 minutes. During incubation, gently invert the tubes 3–5 times to ensure thorough mixing.	Transfer the sample to a thermomixer, heat block, or water bath and incubate at 70°C for 10 minutes.	Add 5 µl of RNase A to the sample and gently invert the tube 2-5 times. Incubate at room temperature for 3 minutes.
5.	Bring the sample to room temperature for 3 minutes.	Bring the sample to room temperature for 3 minutes.	Bring the sample to room temperature for 3 minutes.	Bring the sample to room temperature. Add 300 µl of HMWB Buffer and 5µl of RNase A. Invert the tube 5 times and incubate for 3 minutes.	Add 300 µl of HMWB Buffer to the sample. Pulse vortex for 1 second (max setting) or invert the tube 5 times to mix thoroughly.

6. Add 400 µL of 100% ethanol and 10 µL of Auto-Mag® HD-1 to the sample. Gently invert the tube 2–5 times to mix thoroughly.

   Note: Complete resuspension of the Auto-Mag® HD-1 beads is crucial for achieving optimal DNA purity.

7. Place the sample on a Mini-Tube Rotator and rotate at room temperature for 10 minutes. Formation of bead–DNA aggregates during rotation is normal; do not attempt to break up or disperse the aggregates.

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

8. Place the samples on a compatible magnetic device for 5 minutes or until the beads are completely cleared from the solution. Carefully remove and discard the supernatant without disturbing the magnetically collected beads.

9. Add 600 µL of Wash Buffer 1 and place the sample on a Mini-Tube Rotator for 5 minutes. After incubation, briefly centrifuge the tube in a mini centrifuge for 2 seconds if any bead aggregates or droplets are attached to the tube cap.

   Note: Wash Buffer 1 must be diluted with ethanol prior to use.

10. Place the sample on the magnet for 1 minute or until the beads are completely cleared from the solution. Carefully remove and discard the supernatant without disturbing the magnetically collected beads.

11. Repeat Steps 9-10 for a second Wash Buffer 1 wash.

12. Add 600 µL of Wash Buffer 2 and place the sample on a Mini-Tube Rotator for 5 minutes. After incubation, briefly centrifuge the tube for 2 seconds if any bead aggregates or droplets are attached to the tube cap.

    Note: Wash Buffer 2 must be diluted with ethanol prior to use.

13. Place the sample on the magnet for 1 minute or until the beads are completely cleared from the solution. Carefully remove and discard the supernatant without disturbing the magnetically collected beads.

14. Repeat Steps 12-13 for a second Wash Buffer 2 wash.

15. Keep the sample on the magnet with the cap open and air-dry the magnetic beads at room temperature for 5 minutes. Carefully remove any residual liquid using a pipette.

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

16. Add 50~150 µl DNA Elution Buffer to the sample. Flick sample tube 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 pipetting will shred the DNA.

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

18. Place the sample back on the magnet for 5 minutes or until the 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 (clear supernatant) to an appropriate storage tube. Store the purified DNA at 4°C for short-term storage or immediate downstream applications. For long-term storage, store the DNA at −20°C.

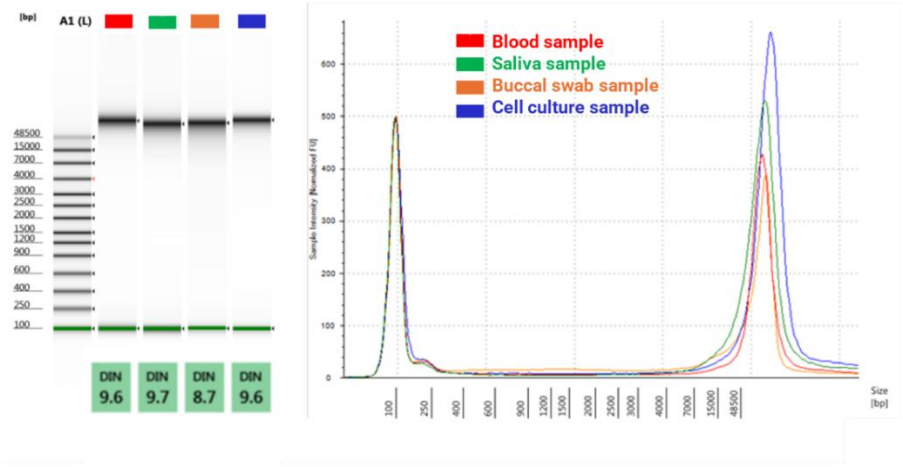
    Note: Do not freeze and thaw HMW DNA repeatedly, it will disrupt the DNA.

20. Additionally, a small amount of clear, gel-like material may remain adhered to the beads after the elution incubation in Step 18. This material is DNA. Allow the sample to rest at room temperature overnight to facilitate complete DNA solubilization. Any visible gel-like material should gradually disperse during this period. After the overnight incubation, gently pipette the sample up and down 5 times, then repeat Steps 17–19 to recover the remaining DNA.

Troubleshooting Tips

- **Low DNA yields:** Ensure 100% Ethanol was added to Wash Buffer 1 or Wash Buffer 2. Use good quality samples, such as fresh blood and saliva; Resuspend Auto-Mag® HD-1 beads by vortex vigorously before use; avoid disturbing the Auto-Mag® beads during aspiration of supernatant; increase elution volume and incubate at 55°C for 10 minutes.
- **Magnetic beads unclear in solution:** Shorter magnetic time can cause beads to remain in solution, increase collection time on the magnetic stand.
- **Downstream applications are unsuccessful:** Could be caused by insufficient DNA in starting materials, so use more starting material. This could also be caused by ethanol carry-over, ensuring that the magnetic beads are completely dry prior to elution.

Supporting Data



Figures 1 illustrates the performance of HMW gDNA isolated from whole blood, saliva, cultured cell culture and samples with Auto-Mag® HMW DNA Isolation Kit. The isolated HMW DNA was analyzed on Agilent's TapeStation 2200.

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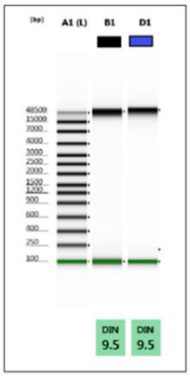

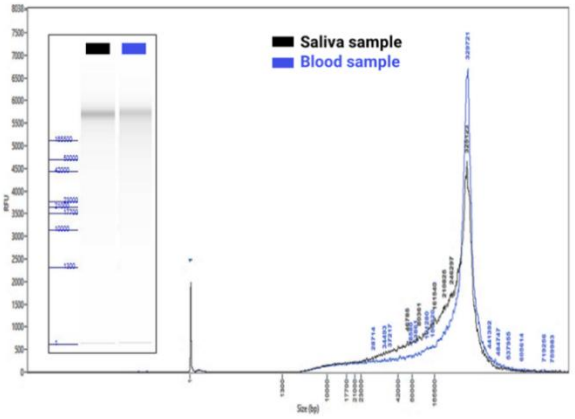

Figures 2 illustrates the analysis results of HMW gDNA extracted from blood and saliva samples on TapeStation and Femto Plus. The left figure shows the results on TapeStation: both gDNA samples have good quality (DIN=9.5). The right figure shows the results on Femto Pulse: over 85% of the DNA is over the peak size of 50kbp, while the main peak is around 320kbp. The results support that the Auto-Mag HMW DNA Isolation Kit can produce high-quality HMW DNA for sequencing and genetic analysis.

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HMW DNA Size Selection

Auto-Mag® HMW Size Select

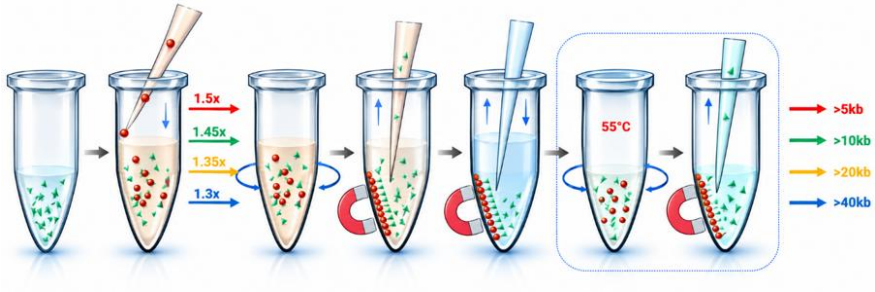
Catalog Number: S005-00, S005-01, S005-02

Introduction

Auto-Mag® HMW Size Select is a new and truly practical magnetic bead-based reagent for short DNA fragment depletion and High Molecular Weight (HMW) DNA size selection and quickly cleanup. The strategies of the HMW DNA size selection is to remove confounded <5kb, <10kb, <20kb or longer DNA fragments from HMW DNA samples by simply adjusting the volume ratio of reagents to samples and recover the larger DNA fragments adsorbed on the magnetic beads. This solves the DNA quality requirements for library construction on third-generation sequencing platforms and significantly improves the average read length of third-generation sequencing platforms (such as PacBio SMRT technology or Oxford Nanopore technology).

The Auto-Mag® HMW Size Select reagent can be used for unsheared HMW DNA cleanup and be used to selectively recover desired sized HMW DNA fragments from fragmented gDNA samples or even partially degraded gDNA samples or larger PCR amplicon products. Auto-Mag® HMW Size Select uses magnetic beads and requires no centrifugation or filtration, it is suitable for manual processing as well as adapting to automatic liquid handling workstations.

Workflow



Sample Information and Pre-Prepare

Long-read sequencing requires high-quality, high molecular weight (HMW) DNA. Before performing DNA cleanup, it is necessary to evaluate the quantity and size distribution of input DNA to determine whether it is suitable for the protocol

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- To isolate HMW genomic DNA with a qualify HMW DNA isolation kit and 50% of HMW DNA should be >30 kb.
- DNA concentration should be measured using a Qubit dsDNA Broad Range Assay or equivalent and concentration is between 10–150 ng/µl. If the DNA concentration is over 150ng/µl, dilute sample using TE buffer (pH 8), Elution Buffer, or water.
- Unsheared genomic DNA sample should be in DNA Elution buffer, TE buffer (pH 8.0) or water and concentration is between 10–150 ng/µl. If you are unsure of the buffer composition or if the gDNA is not in Elution Buffer, perform a 1X Auto-Mag® X-Pure Size Select purification followed by elution with Elution Buffer or an equivalent low salt buffer (i.e., TE Buffer, 10 mM Tris-HCl, pH 8.0, or water)
- The sheared genomic DNA sample should be in low Ionic strength buffers. If not, perform a 1X Auto-Mag® X-Pure Size Select purification followed by elution with Elution Buffer or an equivalent low salt buffer (i.e., TE Buffer, 10 mM Tris-Cl, pH 8.0, or water). The concentration of fragmented DNA can be between 5–150 ng/µl.
- For partially degraded genomic DNA samples, DNA samples should first be purified by using 1X Auto-Mag® X-Pure Size Select and eluted in Elution Buffer or an equivalent low salt buffer (i.e., TE Buffer, 10 mM Tris-HCl, pH 8.0, or water). The concentration can be adjusted to 5–150 ng/µl.
- Sample volume: Although the starting sample volume can be any volume greater than 10ul. However, the size range of recovered gDNA is sensitive to changes in the volume ratio of reagent to sample. Using a smaller sample volume may increase the chance of size shift of recovered DNA by sampling error. It is recommended to use 50µl of sample. If a smaller sample volume must be used, please refer to the volume ratio of reagent to sample corresponding to the size range of recovered DNA and use a more accurate pipette.

Processing Tips

Pipetting

When adding, mixing sample, use wide bore pipette tips. Do not disturb beads when adding or removing liquid from the microcentrifuge tube or well of plate. Carefully insert the pipette tip against the wall and on opposite side towards the beads to dispense, or remove liquid as shown in Figure.

Wide bore tips

If there are no wide bore tips to mix. Do not vortex, cut the regular tips to resemble wide bore

Heterogeneity and viscosity

Recovery efficiency and size selection performance of Auto-Mag® HMW Size Select depend on the input DNA being homogeneous and fully in solution. HMW DNA may be difficult to re-solubilize after extraction and results in an inhomogeneous sample. Such samples will result in low yields and carry-over of short DNA if used with the Auto-Mag® HMW Size Select reagent. If the sample is inhomogeneous or contains viscous jellies, we recommend needle shearing with a 26G needle and then allowing the DNA to rest at room temperature overnight before beginning size selection.

HMW DNA Size Selection Protocols

This procedure is suitable for removing confounded or unwanted small DNA molecules from unsheared high molecular weight (HMW) gDNA, sheared gDNA fragments, or partially degraded gDNA samples. By adjusting the ratio of reagents to samples, the desired size ranges of HMW DNA fragments can be recovered. The recovered HMW DNA is also cleaned during the process which could help remove other contaminants from samples.

1. Completely float the Auto-Mag® MHW Size Select reagent until it appears homogeneous in color.
2. Pulse vortex or pipette mix each sample to homogenize the DNA in solution. Adjust the DNA sample to a total volume of 50µl and DNA concentration between 10–150 ng/µl.

   Note: Quick spin each sample to collect liquid if necessary.
3. Pipette sample into a 1.5 ml microcentrifuge tube, or the well of 96 plate (96 well format)

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4. Refer to the table below and add an appropriate volume of Auto-Mag® MHW Size Select reagent to 50ul of HMW gDNA sample to selectively deplete the short size DNA fragments and recover DNA fragments of the desired size.

   *Note: For example: for depletion of fragment range **<10 kb** and recovery DNA fragments >10 kb from the **50ul** of sample, add **72.5ul (1.45x)** of Auto-Mag® MHW Size Select reagent to the **50ul** of sample.*

Size range of short DNA to be removed	Ratio of Auto-Mag® MHW Size Select / Sample	Vol. of Auto-Mag® MHW Size Select Needed for 50ul of sample
< 5 kb	1.5 x	75µl
< 10 kb	1.45x	72.5µl
<20kb	1.35x	67.5µl
<30kb	1.3-1.25x*	65-62.5µl*
When using Auto-Mag® HMW Size Select, the reagent-to-sample volume ratio is critical for achieving the desired DNA fragment size range and recovery yield. This is particularly important when selecting DNA fragments larger than 30 kb, as even slight variations in the volume ratio may affect both the size distribution and yield of the recovered HMW DNA. To achieve optimal results, we recommend following the parameters provided in the table above, performing preliminary tests with a small number of samples, and making minor adjustments to the reagent-to-sample volume ratio as needed to obtain the desired DNA fragment size range and recovery yield.		

5. Mix the sample by gently pipetting up and down 15 times with wide bore pipette tips.

   Note: It is important to mix well.

6. Incubate sample at room temperature for at least 15 minutes for maximum recovery.

7. Place the sample tubes or plate on a compatible magnetic separation device for 10 minutes or until the beads are completely cleared from solution. Beads will pull to the side of the tube or wells,

   Note: Magnetic separation time may be related to the strength of the magnetic separation device and the amount of sample. If necessary, gently pipette up and down twice to speed up magnetic bead separation. Do not touch the magnetic beads on the sides of the tube or wells.

8. Keep the sample on the magnet, Carefully Pipette out and discard all liquid which contains short DNA fragments. Do not disturb the beads.

   Note: There is unbound short DNA in the supernatant. If unbound DNA needs to be recovered, transfer supernatant to new tube or 96-well plate. Reference the protocol of unbinding short DNA fragments to recover the DNA fragments.

9. With the samples still on the magnet, slowly add 200µl of 80% ethanol to each sample and gently pipette up and down twice. Incubation for 30 seconds. Carefully Pipette out and discard all liquid. Do not touch the magnetic beads.

   Note: 80% ethanol is hygroscopic and should be prepared FRESH to achieve optimal results.

10. Repeat step 9 for two more washes.

11. 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 traces of alcohol but take caution in not over drying the beads as this will significantly decrease elution efficiency.

12. Remove the samples from the magnet. Add 20-100µl of Elution buffer and ensure the beads are submerged in the Elution buffer Do not mix the sample with a pipette or vortex. incubate the sample at 55°C for 5 minutes first.

    Note: Elution Buffer volume may be adjusted to achieve desired concentration. Longer DNA fragments can take more time to release from beads. eluting for more time can help increase recoveries.

13. After 5 minutes incubation, gently pipetting up and down elution buffer 10 times with wide-bore pipette tips to ensure the beads are properly re-suspended in the elution buffer. Incubate sample again at room temperature for an additional 5 minutes.

    Note: When resuspending magnetic beads, the volume of the pipettes should be adjusted to be smaller than the volume of Elution buffer to prevent bubbles generated when suspending magnetic beads.

14. Place the sample back on the magnet for 5 minutes or until the beads are completely cleared from Elution buffer.

15. Transfer the eluate to an appropriate storage vessel and analyze the recovery and purity of the DNA using a

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NanoDrop and Qubit system.

16. The DNA can be stored in Elution Buffer at 4°C for several months. Keep at -20°C for long term storage. Avoid freeze/thaw cycles since this can degrade high molecular weight DNA.

Troubleshooting Tips

- **Low DNA yields:** Recovery will be low if DNA is not HMW. Verify that 50% of input DNA should be ⩾30 kb. Low concentration of input DNA. Try increasing concentration of input DNA up to the maximum of 150 ng/µl; mixing thoroughly during the initial bind mix and elution mix is critical. If beads get aspirated into tips during supernatant removal, the nucleic acid bound to these beads will also be lost. Aspirate slowly and remove as much of the first supernatant as possible without disturbing the bead.
- **Incompatible fragment sizes**: Make sure the pipette is adequately calibrated, the ratio of reagents to samples is very sensitive, so small volume errors may cause a shift in the size selection.
- **Downstream applications are unsuccessful:** Could be caused by insufficient DNA in starting materials, so use more starting material. This could also be caused by ethanol carry-over, ensuring that the magnetic beads are completely dry before elution.

Supporting Data

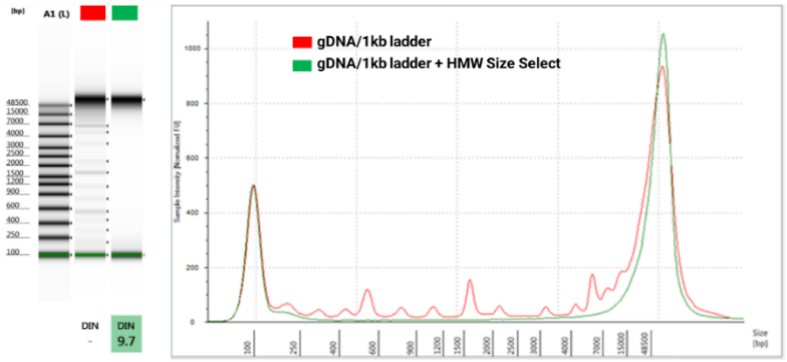
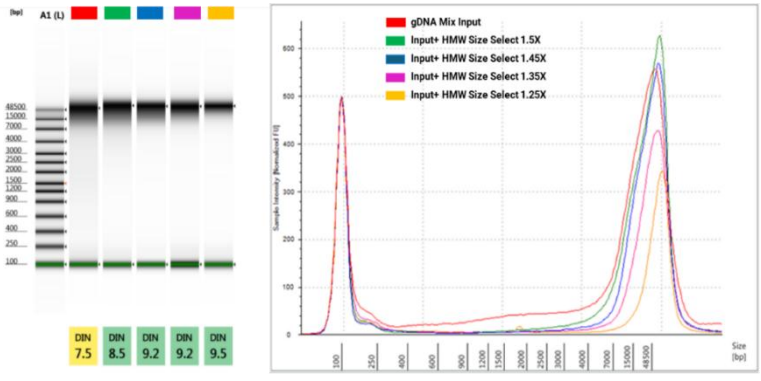


Figure 1: illustrates the performance of the short DNA fragment depletion of Auto-Mag® HMW Size Select on HMW DNA spiked with 1kb+ ladder. All DNA fragments <10 kb in length is removed to trace levels and HMW DNA > 10kb are recovered. Data were analyzed on an Agilent TapeStation 2200.

Figure 2: shows the results of size selection of HMW DNA using Auto-Mag® HMW Size Select. The input gDNA is a mixture of partially degraded gDNA and gDNA extracted from blood. As the volume ratio of Auto-Mag® HMW Size select reagent to DNA sample is reduced, the magnetic beads gradually lose the ability to bind smaller DNA fragments, and small DNA fragments in the range of <5kb, 10kb, <20kb or longer in the sample can be easily removed, thereby recovering larger HMW DNA fragments. The test results were analyzed on the Agilent TapeStation 2200.



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