Isohelix Mag-Filter HMW Clean-Up Kit HMW-5/HMW-50: Instructions for Use

Product Details:
The Mag-Filter HMW kit utilizes magnetic bead chemistry for the depletion and removal of low molecular weight DNA fragments from purified DNA samples for NGS & long-read sequencing applications, and for the enrichment of high quality, high purity long-read DNA.

- Efficient removal of short-read DNA.
- Fully scalable kit for high throughput of samples.
- Maximises sample purity.
- Fast sample prep, avoiding lengthy gel separation methods.

Kit Contents:

Catalogue Number	HMW-5	HMW-50	Storage Temperature
No. of samples processed	5 x 100ul clean-ups	50 x 100ul clean-ups	
HMW Binding Buffer (BBH)	500 µl	5 ml	Room Temperature
Wash Buffer (WB)	1.5 ml[1]	15 ml[2]	Room Temperature
Elution Buffer (EB)	500 µl	5 ml	Room Temperature
Magnetic Beads (MB)	5 µl	50 µl	+4°C

(1) Add 1.32ml >98% Molecular Biology grade Ethanol into solution WB before first use, tighten the cap securely when not in use to prevent evaporation.
(2) Add 13.2ml >98% Molecular Biology grade Ethanol into solution WB before first use, tighten the cap securely when not in use to prevent evaporation.

Storage:
Isohelix DNA Kits are shipped at ambient temperature.
Please note that on arrival the kit components should be stored according to the table above.
The kits are stable up to the expiry date if stored as instructed. See box label for expiry date.

Required Equipment (Supplied by User):

- Magnetic separation rack for 1.5ml/2.0ml microtubes, or a 96-well format magnetic rack compatible with PCR microplates for high-throughput processing.
- Micropipettes (P200/P1000) with wide-bore pipette tips. 8-channel pipettes are recommended for high throughput processing.
- Ethanol (≥98% Molecular Biology Grade).
- Heating Block pre-heated to 60°C.
- Vortexer.

Note on Handling High Molecular Weight DNA:

HMW DNA can be sensitive to mechanical shearing which will reduce the efficiency of the kit. Follow the following tips to maximise sample quality:

I. Use wide-bore tips for pipetting and mixing steps. When mixing samples use a gentle and slow pipetting technique. Avoid mixing samples by vortexing.
II. If using frozen samples, ensure that they are fully thawed out before use. Avoid use of samples that have undergone multiple freeze-thaw cycles.

This kit has been designed for Research Use Only

Important Note Before Starting:

Ensuring that your input DNA is of high-quality is vital for the full functioning of this kit, therefore it is highly recommended to QC samples for yield and quality before use. Recommended DNA input is **≥50ng/µl** as measured using fluorometric assay, and the presence of HMW DNA should be confirmed by high-resolution electrophoresis methods. Low-purity (<1.7 A260/280) samples can be used as the kit will remove contaminants. **Input of low-quality DNA will result in poor recoveries.**

Microtube Clean-Up protocol for 100µl of purified DNA Sample in TE or Tris Buffer:

1. Aliquot 100µl of each DNA sample in separate 1.5ml micro tubes (or into a 96 well PCR plate if using a 96-well format magnetic rack). Pre-heat the Elution Buffer EB to 60°C on the heating block. Mix Magnetic Beads thoroughly by vortexing before use.

2. Prepare a sufficient amount of DNA Binding Buffer/Bead Mix appropriate for the number of samples to be processed. When preparing binding mix for multiple samples, multiply the volume per sample for each reagent by the number of samples to be processed and add a 10% overage. The table below can be used as a guide:

Reagent	Volume Per Sample	Volume for 5 Samples	Volume for 50 Samples
Binding Buffer (BBH)	69 µl	380 µl	3795 µl
Magnetic Beads (MB)	1 µl	6 µl	55 µl
Total	**70 µl**	**386** µl	**3850 µl**

3. Add 70µl of the Binding Buffer/Bead Mix to each sample and mix well by carefully pipetting up and down ten times. Incubate samples at room temperature for at least 5-10 minutes to allow the DNA to bind to the beads.

4. Place sample tubes/plate on the magnetic rack for 5 minutes to allow beads to collect against the magnets.

5. Without removing the sample tubes/plate from the magnets, carefully remove and discard the supernatant from each sample without disturbing the beads.

6. Remove samples from the magnetic rack and add 200µl of Wash Buffer (WB) to each sample, pipetting up and down carefully five times to mix. Replace the samples on the magnetic rack for one minute, after which carefully remove and discard the supernatant.

7. Repeat Step 6 once more using 100µl Wash Solution.

8. Dry the excess Wash Buffer from the beads by leaving samples uncapped for 30 seconds on the magnetic rack. Remove any remaining buffer after this time using a pipette.

9. Remove samples from the rack and add 50-100µl of pre-heated Elution Buffer (EB) to each, pipetting up and down carefully to mix. Cap the samples and leave to stand for 5 minutes to elute the DNA from the beads.

10. Following incubation, place the samples back on the magnetic rack for at least 5 minutes to allow the beads to separate from the purified DNA. Transfer the supernatant containing the purified DNA to a fresh 1.5ml tube or plate.

    Optional: If samples appear turbid after elution, they can be briefly centrifuged for 1 minute, after which the supernatant can be transferred to a new 1.5ml tube or plate.

Purified DNA is ready for use immediately and can be stored for up to a week at +4°C or at -20°C for long term storage.

Technical Assistance:
If you have any questions regarding the use of this kit or other Isohelix products please contact us by email at info@isohelix.com or for further information visit the website at www.isohelix.com

Safety and Use of the Isohelix DNA kits:

Buffers in the Isohelix DNA kits contain irritants so appropriate safety equipment such as gloves, laboratory coats and eye protection should be worn. The kits are intended for use by qualified professionals trained in potential laboratory hazards and good laboratory practice. If direct information is not available on any of our compounds this should not be interpreted as an indication of product safety.

This kit has been designed for Research Use Only