MAGBIO

"WE MAKE NGS BETTER"

Short Fragment Depletor - 10HT

Progressively Depletes Short DNA Fragments <10 kb

Catalog Nos. SF-80001, SF-80005, SF-80050
Manual Revision 2
WI-72-135

- Magnetic bead-based size selection of DNA fragments >10 kb

Protocol

Contents

For research use only

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Product Description

Short DNA fragments are problematic in next generation sequencing because if sequenced, it may not be possible to map the short reads to a specific region of the reference genome and the data may be discarded. The Short Fragment Depletor - 10 High Throughput kit (SFD-10HT) uses bead-based size selection for the removal of DNA fragments <10 kb. This results in the improvement of DNA sample quality for 3rd generation sequencing platforms which require long dsDNA input samples for long reads that can be easily mapped.

There are cases where the starting sample material is of lower quality, where DNA material may have been partially degraded due to sub-optimal storage. Also, some sample matrices are difficult to lyse, and they tend to contain a mix of both large and short fragments. In both cases, it is necessary to carry out large fragment (>10 kb) enrichment steps for better sequencing results. The Short Fragment Depletor -10 High Throughput kit can be used in both sample types to remove the short fragments prior to library prep.

The kit is tolerant to a wide range of DNA concentrations and works optimally in the range of 0.5-150 ng/µL. It has a very high recovery efficiency (50-90%) depending on the input sample quality.

Process

DNA amplicons and fragments >10 kb are selectively bound to the magnetic particles; and highly purified DNA is eluted with low salt elution buffer or water which can be used directly for downstream applications. SFD-10HT is compatible with some of the 3rd next generation sequencing platforms. This product can be used manually or in automation.

Benefits

- Size selection to progressively deplete short fragments <10 kb in a sample
- Improvement in the read length
- Can be used with HMW DNA, fragmented DNA, sheared DNA, and PCR amplicons

Materials Supplied in the Kit

- Short Fragment Depletor - 10HT

Kit Contents

Short Fragment Depletor - 10HT Catalog No.	**SF-80001 (Sample)**	**SF-80005**	**SF-80050**	**Storage**
Short Fragment Depletor - 10HT volume	400 µL	5 mL	50 mL	2-8°C

Shipping and Storage

- SFD-10HT ships at room temperature. Store at 2-8°C.

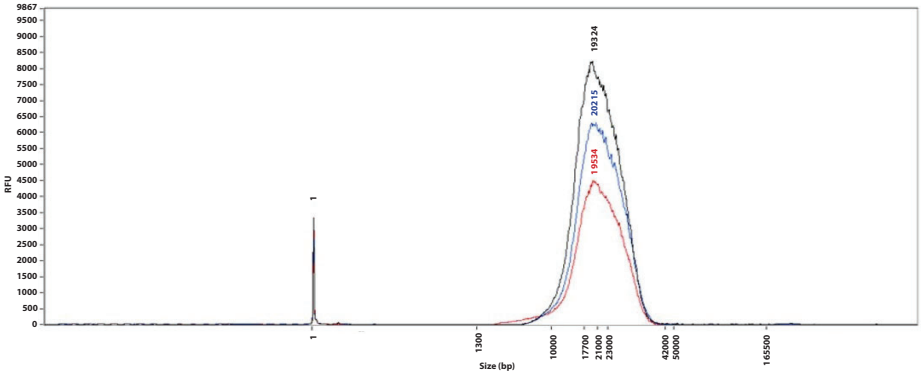
Safety Information

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.

Expected Results

The improvement in size selection or removal of short fragments depends on the quality of input DNA as shown below.

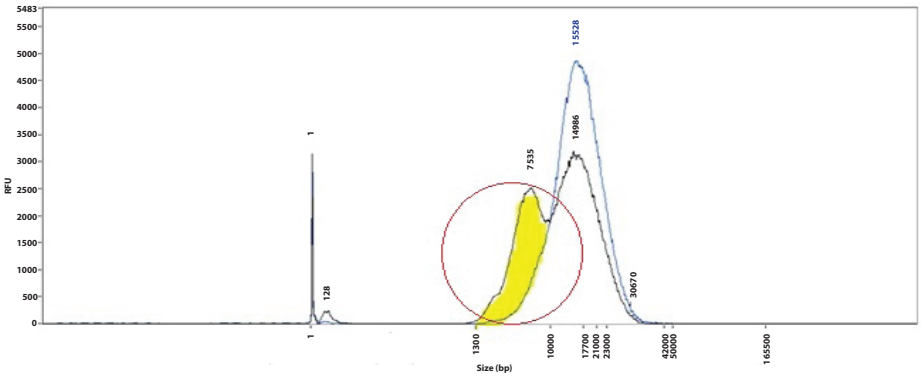
Smear Analysis: High Quality Sheared DNA



Sample	Range	ng/uL	% Total	nmol/L	Avg. Size	Size Threshold (b.p.)	DQN
Selection (0.40x)	10,000 bp to 300,000 bp	0.3623	97.8	0.028	21,275	10,000	9.8
Input DNA	10,000 bp to 300,000 bp	0.2478	95.4	0.0193	21,171	10,000	9.5

The quality of this input sample is already good. Fragments that are >10 kb make up to 95.4% in total before selection. Therefore, selection with SFD-10HT is not going to show drastic difference as shown by the smear analysis results above. The smear analysis at 0.40x (suggested selection ratio) shows an improvement of around 2%. 0.30x and around 97% for 0.35x selection. The selection will never be 100%, SFD-10 HT facilitates near removal of less than 10 kb fragments. The sample input quality determines how drastic the selection will look on Femtopulse electropherogram.

Smear Analysis: Poor Quality DNA: 15-20 kb fragments with 7-8 kb Fragments



Sample	Range	ng/uL	% Total	nmol/L	Avg. Size	Size Threshold (b.p.)	DQN
Selection (0.40x)	10,000 bp to 300,000 bp	0.279	84.4	0.0267	17,180	10,000	8.4
Input DNA	10,000 bp to 300,000 bp	0.1981	55.8	0.0194	16,837	10,000	5.6

The quality of the sample is not very good, about 44.2% of fragments are below 10 kb and 55.8% of fragments are above 10 kb. Upon SFD-10HT treatment, the sample quality improves from 55.8% to 84.4 and this is also shown by the DQN number improvement from 5.6 to 8.4. SFD-10HT shows drastic improvement of sample quality.

Protocol:
Short Fragment Depletion of DNA Fragments <10 kb (1.5/2.0 mL tube or 96 well format)

Equipment and Reagents to Be Supplied by the User

- ☐ Thermomixer or incubator
- ☐ 1.5 mL LoBind microcentrifuge tubes/96 well PCR plate
- ☐ 80% Ethanol (Prepared from non denatured Ethanol)
- ☐ Qubit fluorometer and reagents for DNA concentration measurement

Protocol

⚠ Input DNA concentration must be in the range of 0.5-150 ng/µL to attain high recovery efficiency. If the DNA concentration is above 150 ng/µL, dilute the sample before performing the experiment.

⚠ Bring the **SFD-10HT** to room temperature for at least 30 minutes before use.

1. Transfer the PCR reaction/DNA sample to an appropriate 96 well PCR plate or 1.5-2 mL LoBind DNA tube.

   ⚠ *If the sample volume is less than 50 µL, adjust the volume by adding elution buffer or sterile water.*

2. Shake the **SFD-10HT** thoroughly to fully resuspend the magnetic beads.

3. Add **SFD-10HT** volume according to the reaction table below. Use a 0.40x bead ratio for selection of fragments >10 kb or for depletion of fragments <10 kb.

   See the table below to determine the appropriate volume

PCR Reaction Volume (µL)	SFD-10HT Volume at 0.40x (µL)*
50	20
100	40
150	60

*Formula used to calculate the volume of SFD-10HT needed per reaction:
SFD-10HT volume per reaction = 0.40 X PCR reaction volume or DNA sample volume.

4. Thoroughly mix the **SFD-10HT** and DNA sample by mix-pipetting up and down 10-15 times using wide-bore pipette tips.

5. Incubate the mixture for 1 hour at room temperature.

6. Place the sample plate/tube on the magnetic separation device for 3 min or until the solution clears. The beads will pull to the side of the well.

7. With the sample plate/tube still on the magnet, remove and discard the supernatant by pipetting.

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

8. With the sample plate/tube on the magnet, add 200 µL of 80% Ethanol to each well/tube and incubate for 30 seconds at room temperature.

9. With the plate/tube still on the magnet, remove and discard the supernatant by pipetting.

10. Repeat steps 8-9 for a total of two 80% Ethanol washes.

11. Check to make sure that there is no remaining 80% Ethanol in the sample well/tube. If droplets are present, do the following:

    i. Spin the sample tube or plate down to bring the droplets down
    ii. Place the sample on the magnetic separation device
    iii. Use a fine pipette tip to remove the droplets without touching the beads

12. Dry the beads by incubating the plate for 1-2 minutes at room temperature with the plate still on the magnetic separation device.

    ⚠ *It is critical to completely remove all traces of alcohol, but take caution in not over drying the beads as this will break the DNA fragments.*

13. Remove the sample plate/tube from the magnetic separation device. Add 50 µL of elution buffer (reagent grade water, TRIS-HCl pH 8.0 or Buffer EB) to each well/tube and pipette up and down 5 times to mix.

    ⚠ *Use the desired elution volume.*

14. Perform warm elution by heating the sample plate/tube at 37°C for 15 minutes to elute the DNA from the beads.

15. Briefly spin down the sample and place the sample plate/tube on a magnetic separation device. Wait for the solution to be clear and pipette out the supernatant to a new 1.5 mL LoBind tube. Place the sample plate/tube back on the magnetic separation device and wait 3 minutes or until the magnetic beads clear from the solution.

16. Transfer the eluate (cleared supernatant) to a new plate/tube for storage or for subsequent applications.

Quick Guide

1. Add 50 µL of DNA sample and 20 µL of SFD-10HT. Mix 10-15 times.

2. Let the mix sit for 1 hour at room temperature.

3. Magnetize the beads and discard the supernatant when the solution looks clear.

4. Perform 2 bead washes with 80% Ethanol.

5. Do heated elution at 37°C for 15 min (use the desired elution volume).

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
Short fragments percentage remains high after selection Large fragments are not enriched	Incorrect sample or bead volume	Measuring precise sample and bead volume is critical to obtaining good results. Make sure to add the correct volume of SFD-10HT according to the reaction table on page 2
	Sample heterogeneity	It is critical to thoroughly mix the sample and beads to achieve successful size-selection
	Beads left on the magnet for too long to dry	When drying the beads at the last step before elution, do not over dry the beads because as the beads crack so does the DNA contained in the bead
Low DNA recovery	Incorrect sample tubes used that bind DNA to the walls of the tube	It's important to use LoBind tubes to avoid DNA binding to the tube surface. This has an impact in DNA recovery
	Ethanol used is not fresh	Use freshly prepared 80% Ethanol at all times for the washes. Ethanol has to be prepared fresh every time to have the best results. Ethanol can easily evaporate during storage, so be sure to tighten the lid when closing Ethanol bottles
There is no removal of short fragments	Input concentration is higher than the recommended DNA concentration range	If the sample is out of the functional range of SFD-10HT, then consider diluting the sample to the acceptable volume range (0.5 ng/µL to 150 ng/µL)
DNA quantitation readings are inconsistent	The technology used to measure DNA is not very specific to DNA measurement	For quantification of DNA input, consider using a fluorometer such as Qubit. Fluorometers provide accurate, specific, and sensitive quantification of DNA.

Ordering

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)	

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)	

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

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)