

Nanobind[®] HT kits

Guide & overview

For extraction of HMW (50–300 kb) genomic DNA from cultured cells, 200 μ L blood samples, and 1 mL blood samples.

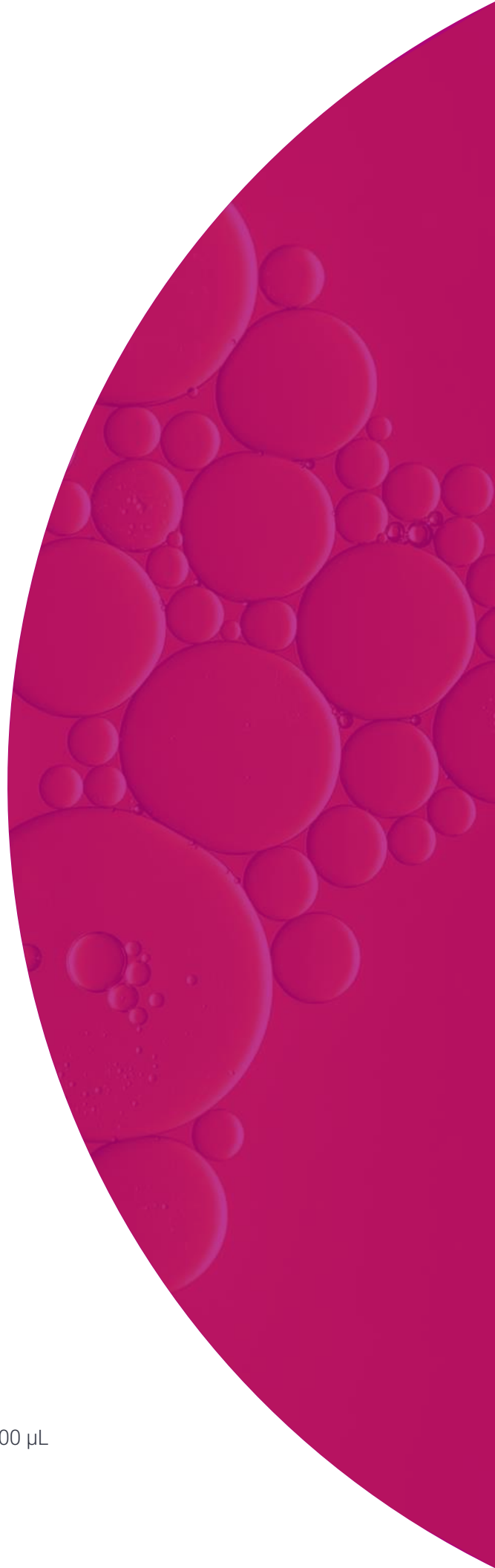


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Prior to starting

Buffer CW1 and CW2 are supplied as concentrates. These kits use CW1 and CW2 with 60% final ethanol concentrations. Before using, add the appropriate amount of ethanol (96–100%) to Buffer CW1 and Buffer CW2 as indicated on the bottles.

Kit storage

RNase A should be stored at 4°C upon arrival.

Nanobind disks and all other buffers should be stored at room temperature (15–30°C).

Safety precautions

Buffer BL3 and Buffer CW1 contain guanidine hydrochloride. Warning! Guanidine hydrochloride is harmful if swallowed or inhaled and causes skin and eye irritation. DO NOT mix with bleach or acidic solutions.

Product use

Nanobind HT kits are intended for research use only.

Required materials and equipment

Nanobind HT CBB

Equipment/reagents needed for all platforms	Manufacturer (part number)
Nanobind HT CBB kit	PacBio® (102-762-700)
Ethanol (96–100%)	Any major lab supplier (MLS)
Isopropanol (100%)	Any MLS
1X PBS	Any MLS
UV/Vis	Thermo Fisher Scientific NanoDrop 2000
Fluorescent DNA quantification	Invitrogen Qubit 3.0, dsDNA BR and RNA BR assay kits

KingFisher Apex equipment/consumables	Manufacturer (part number)
KingFisher Apex system	Thermo Fisher Scientific (5400930, includes Apex 96 deep well magnet head)
KingFisher Apex 96 deep-well magnet head	Thermo Fisher Scientific (24079930)
KingFisher Apex 96 deep-well heating block	Thermo Fisher Scientific (24075920)
KingFisher 96 deep-well plates, barcoded	Thermo Fisher Scientific (95040450B)
KingFisher 96 deep-well tip combs, barcoded	Thermo Fisher Scientific (97002534B)

KingFisher Duo equipment/consumables	Manufacturer (part number)
KingFisher Duo Prime system	Thermo Fisher Scientific (5400110)
KingFisher 96 deep-well plates	Thermo Fisher Scientific (95040450)
KingFisher 12-tip comb, for 96 deep-well plate	Thermo Fisher Scientific (97003500)
KingFisher elution strip for 12-pin magnet	Thermo Fisher Scientific (97003520)

KingFisher Flex equipment/consumables	Manufacturer (part number)
KingFisher Flex system	Thermo Fisher Scientific (5400630, includes 96 deep well magnet head)
KingFisher Flex 96 deep-well magnet head	Thermo Fisher Scientific (24074430)
KingFisher Flex 96 deep-well heating block	Thermo Fisher Scientific (24075430)
KingFisher 96 deep-well plates	Thermo Fisher Scientific (95040450)
KingFisher 96 tip combs for deep-well magnets	Thermo Fisher Scientific (97002534)

Hamilton NIMBUS Presto equipment/consumables	Manufacturer (part number)
NIMBUS Presto assay ready workstation	Hamilton Company
KingFisher Presto 96 deep-well head	Thermo Fisher Scientific (24078830)
KingFisher 96 deep-well plates	Thermo Fisher Scientific (95040450)
KingFisher 96 deep-well tip comb for deep-well magnets	Thermo Fisher Scientific (97002534)
60 mL reagent reservoir	Hamilton Company (56694-01)
200 mL reagent reservoir	Hamilton Company (56695-01)
1000 µL conductive filter tips	Hamilton Company (235905)
300 µL conductive filter tips	Hamilton Company (235903)
300 µL wide bore 0.71 mm orifice conductive filter tips	Hamilton Company (235452)
Screw cap micro tube, 2 mL	Sarstedt Inc (72.694.406)

Nanobind HT 1 mL blood

Equipment/reagents needed for all platforms	Manufacturer (part number)
Nanobind HT 1 mL blood kit	PacBio (102-762-800)
Ethanol (96–100%)	Any MLS
Isopropanol (100%)	Any MLS
UV/Vis	Thermo Fisher Scientific NanoDrop 2000
Fluorescent DNA quantification	Invitrogen Qubit 3.0, dsDNA BR and RNA BR assay kits

KingFisher Apex equipment/consumables	Manufacturer (part number)
KingFisher Apex system	Thermo Fisher Scientific (5400940, includes Apex 24 Combi magnet head)
KingFisher Apex 24 Combi magnet head	Thermo Fisher Scientific (24079940)
KingFisher Apex 24 deep-well heating block	Thermo Fisher Scientific (24075940)
KingFisher 24 deep-well plates, barcoded	Thermo Fisher Scientific (95040470B)
KingFisher Apex 24 deep-well tip comb & plates, barcoded	Thermo Fisher Scientific (97002610B)
































KingFisher Duo equipment/consumables	Manufacturer (part number)
KingFisher Duo Prime system	Thermo Fisher Scientific (5400110)
KingFisher 24 deep-well plates	Thermo Fisher Scientific (95040470)
KingFisher 6-tip comb, for 24 deep-well plate	Thermo Fisher Scientific (97003510)

KingFisher Flex equipment/consumables	Manufacturer (part number)
KingFisher Flex system	Thermo Fisher Scientific (5400640, includes Flex 24 deep-well magnet head)
KingFisher Flex 24 deep-well magnet head	Thermo Fisher Scientific (24074440)
KingFisher Flex 24 deep-well heating block	Thermo Fisher Scientific (24075440)
KingFisher 24 deep-well plates	Thermo Fisher Scientific (95040470)
KingFisher 24 deep-well tip comb & plates	Thermo Fisher Scientific (97002610)

Hamilton NIMBUS Presto equipment/consumables	Manufacturer (part number)
NIMBUS Presto assay ready workstation	Hamilton Company
KingFisher Presto 24 deep-well head with heating block	Thermo Fisher Scientific (24078841)
KingFisher 24 deep-well plates	Thermo Fisher Scientific (95040470)
KingFisher 24 deep-well tip comb & plates	Thermo Fisher Scientific (97002610)
60 mL reagent reservoir	Hamilton Company (56694-01)
200 mL reagent reservoir	Hamilton Company (56695-01)
1000 µL conductive filter tips	Hamilton Company (235905)
300 µL conductive filter tips	Hamilton Company (235903)
300 µL wide bore 0.71 mm orifice conductive filter tips	Hamilton Company (235452)
Screw cap micro tube, 2 mL	Sarstedt Inc (72.694.406)

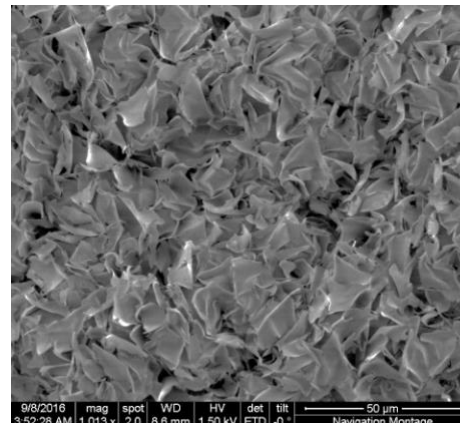
Equipment & consumable quick selection guide

For each automation system, use the following table to select the appropriate magnet head, heating block, plates and tip combs based on Nanobind HT kit.

	KingFisher Duo		KingFisher Flex		KingFisher Apex		Hamilton NIMBUS Presto	
Part	Nanobind HT CBB	Nanobind HT 1 mL blood	Nanobind HT CBB	Nanobind HT 1 mL blood	Nanobind HT CBB	Nanobind HT 1 mL blood	Nanobind HT CBB	Nanobind HT 1 mL blood
Magnet head	KingFisher Duo 12-pin head for 96 DW plates N12459 	KingFisher Duo 6-pin head for 24 DW plates N12460 	KingFisher Flex 96 DW head 24074430 	KingFisher Flex 24 DW head 24074440 	KingFisher Apex 96 DW head 24079930 	KingFisher Apex 24 DW Combi head 24079940 	KingFisher Presto 24 DW head 24078830 	KingFisher Presto 24 DW head 24078840 
Tip comb	KingFisher Duo 12-tip comb for 96 DW plate 97003500 	KingFisher Duo 6-tip comb for 24 DW plate 97003510 	KingFisher 96 tip comb for DW magnets 97002534 	KingFisher 24 DW tip comb & plate 97002610 	KingFisher Apex 96 DW tip combs, barcoded 97002534B 	KingFisher Apex 24 DW tip combs, barcoded 97002610B 	KingFisher 96 tip comb for DW magnets 97002534 	KingFisher 24 DW tip comb & plate 97002610 
Plate	KingFisher 96 DW plate 95040450 	KingFisher 24 DW plate 95040470 	KingFisher 96 DW plate 95040450 	KingFisher 24 DW plate 95040470 	KingFisher 96 DW plates, barcoded 95040450B 	KingFisher 24 DW plates, barcoded 95040470B 	KingFisher DW 96 plate 95040450 	KingFisher 24 DW plate 95040470 
Heating block	KingFisher Duo heating block for 96 DW plate N12461 	KingFisher Duo heating block for 24 DW plate N12462 	96 DW heating block for KingFisher Flex/Presto 24075430 	24 DW heating block for KingFisher Flex/Presto 24075440 	KingFisher Apex 96 DW heating block 24075920 	KingFisher Apex 24 DW heating block 24075940 	96 DW heating block for KingFisher Flex/Presto 24075430 	24 DW heating block for KingFisher Flex/Presto 24075440* 

Introduction

Nanobind technology uses a novel magnetic disk covered with a high density of micro- and nanostructured silica that can be used for rapid extraction and purification of high-quality DNA and RNA. The high surface area and unique binding mechanism give it an extraordinary binding capacity, allowing isolation of high purity, high molecular weight (HMW) and ultra-high molecular weight (UHMW). It uses a standard lyse, bind, wash, and elute procedure that is common for silica DNA extraction technologies. A single disk is used per extraction. However, unlike magnetic beads and silica spin columns which shear large DNA, Nanobind disks bind and release DNA without fragmentation, yielding DNA up to megabases in length.



SEM image of Nanobind's silica surface structure.

Kit overview

The Nanobind HT CBB kit is used for the extraction of HMW (50–300 kb) DNA from cultured cells and 200 µL mammalian blood samples. The Nanobind HT 1 mL blood kit is used for the extraction of HMW DNA from 1 mL mammalian blood samples. The extracted DNA is suitable for HiFi sequencing.

Both the Nanobind HT CBB kit and Nanobind HT 1 mL blood kit support four 24 sample extraction runs (96 samples). The Nanobind HT kits are intended for automated extraction only. They are not compatible with manual extraction.

The [Sample Information](#) section provides example extraction results from a variety of sample types.

Protocols listed in the [Programs](#) section are updated frequently so please check the [PacBio Documentation page](#) for the most up-to-date list and for the current versions of the protocols.

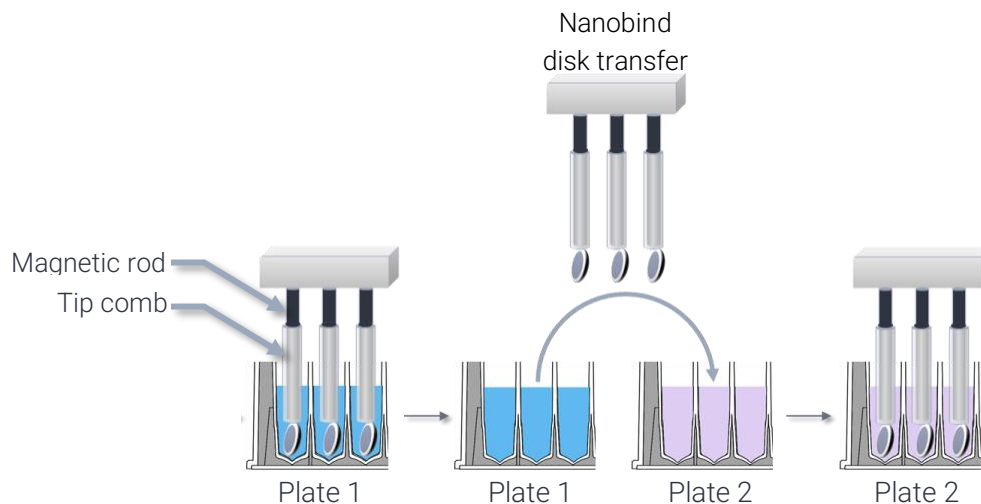
Supported automation platforms for high-throughput HMW DNA extraction using Nanobind HT kits

The procedures are supported on a variety of Thermo Fisher KingFisher instrument. Guidelines on the throughput and approximate workflow times for each supported instrument and procedure are summarized in the table below.

	Thermo Fisher KingFisher Duo Prime			Thermo Fisher KingFisher Flex			Thermo Fisher KingFisher Apex			Hamilton NIMBUS Presto		
Automation level	<ul style="list-style-type: none"> Semi-automated Manual plate filling + requires limited user interaction 			<ul style="list-style-type: none"> Semi-automated Manual plate filling + requires limited user interaction 			<ul style="list-style-type: none"> Semi-automated Manual plate filling + requires limited user interaction 			<ul style="list-style-type: none"> Fully automated Automated plate filling + fully walk-away 		
Workflow (sample #)	1 mL blood (6 samples)	200 µL blood (12 samples)	Cells (12 samples)	1 mL blood (24 samples)	200 µL blood (96 samples)	Cells (96 samples)	1 mL blood (24 samples)	200 µL blood (96 samples)	Cells (96 samples)	1 mL blood (24 samples)	200 µL blood (96 samples)	Cells (96 samples)
Total time	2 hr 1 min	1 hr 26 min	1 hr 17 min	2 hr 12 min	1 hr 45 min	1 hr 36 min	2 hr 12 min	1 hr 45 min	1 hr 36 min	2 hr 30 min	2 hr 35 min	2 hr 23 min
Automation run time	1 hr 50 min	1 hr 13 min	1 hr 4 min	1 hr 50 min	1 hr 13 min	1 hr 4 min	1 hr 50 min	1 hr 13 min	1 hr 4 min	2 hr 10 min	2 hr 10 min	1 hr 58 min
Hands-on time	11 min	13 min	13 min	22 min	32 min	32 min	22 min	32 min	32 min	20 min	25 min	25 min

Nanobind workflow

Nanobind kits use a standard lyse, bind, wash, elute workflow. For automated Nanobind HT extraction kits, each plate (or plate row in the case of the KingFisher Duo Prime instrument) contains a different buffer or sample solution. The Nanobind disk is transferred from one plate to another and mixed.



Sample information

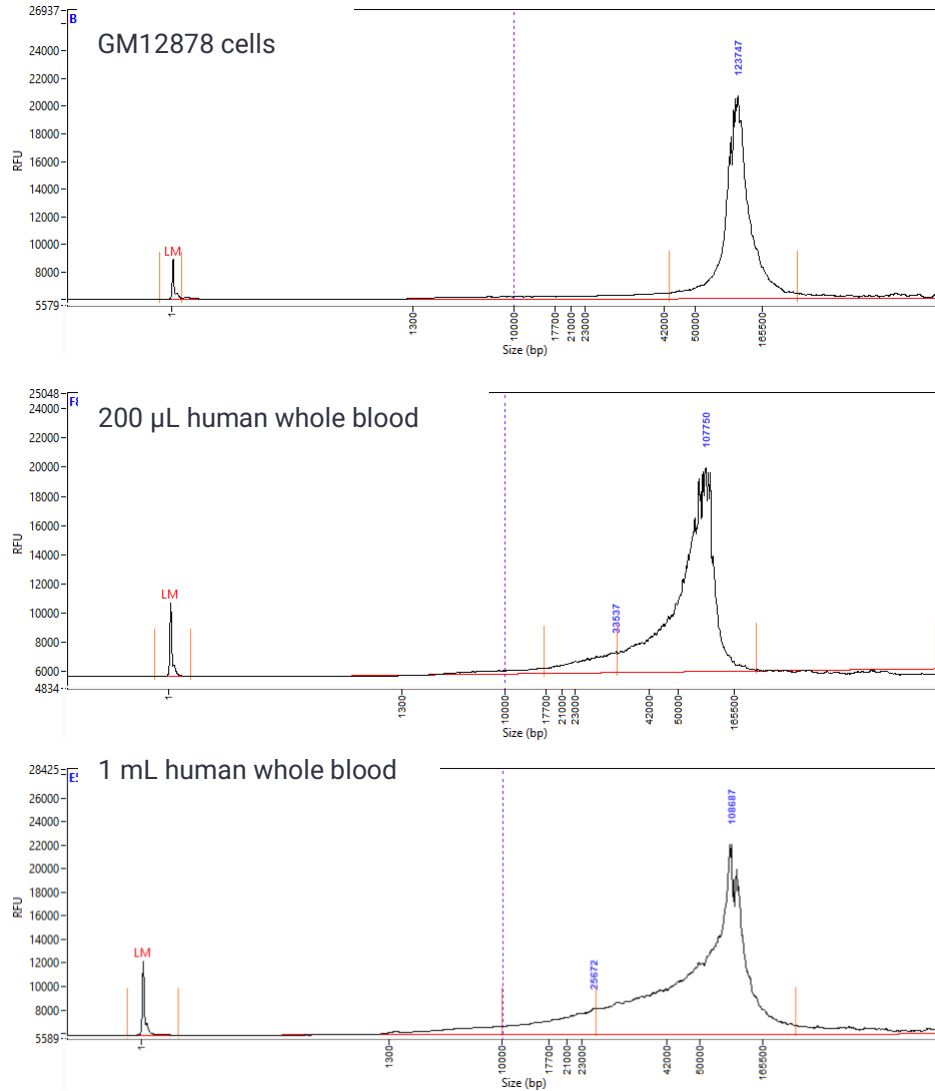
Yields of HMW genomic DNA will vary depending on the sample being processed. The following table provides suggested input ranges and expected yields for the validated sample types on the KingFisher Apex instrument. Comparable results are to be expected between instruments. Each sample type has been validated by HiFi sequencing.

KingFisher Apex example data				
Sample	Suggested input ¹	Example 260/280	Example 260/230	Example yield (µg)
GM12878 (diploid)	1 x 10 ⁶ cells	1.93	2.16	6.16
GM24385 (diploid)	1 x 10 ⁶ cells	1.88	2.18	7.56
MCF-7 (tetraploid)	1 x 10 ⁶ cells	1.95	2.10	14.80
200 µL human whole blood	200 µL	1.83	1.79	6.04
1 mL human whole blood	1 mL	1.89	2.15	32.80

¹Input for cultured cell samples should be adjusted such that DNA yields do not exceed 25 µg. Human whole blood sample input should not deviate from the suggested volumes.

DNA size

The HMW DNA extraction protocols typically yield DNA in the 50–300 kb size range. The exact size will vary depending on sample type, the quality of the starting material, and processing parameters.



Femto Pulse (Agilent) traces for the various sample types extracted on the KingFisher Apex..

Preservation methods

High quality samples are the key to obtaining high-quality DNA. Either fresh or frozen samples can be used equivalently. However, care should be taken to minimize freeze-thaws and to minimize the time samples spend at ambient or at 4°C.

Fresh vs. frozen cells

No systematic difference has been seen in either DNA QC or sequencing results between fresh and frozen cell pellets. Cell pellets should be frozen with minimal media carryover after harvesting. No cryoprotectant is necessary. When using frozen cell pellets, it is important to fully resuspend the cell pellets before processing.

Fresh vs. frozen blood

No systematic difference has been seen in either DNA QC or sequencing results between fresh and frozen blood samples. Blood samples should be frozen as quickly as possible after being drawn. Storage at 4°C should be limited to 2 days from collection to extraction to prevent sample degradation. Blood samples should be aliquoted to avoid repeated freeze-thaws. For frozen blood, we recommend thawing at 37°C for 10–15 min.

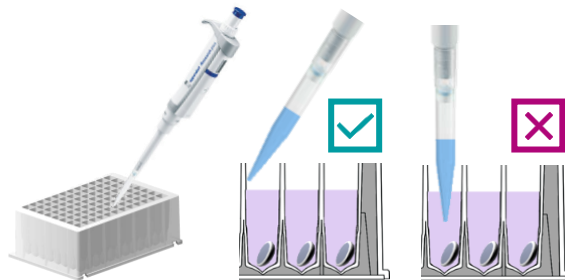
Blood Anti-coagulant

K2 EDTA is the preferred anti-coagulant. Samples stored in sodium heparin (NaHep) and citrate (NaCit) also performed well in very limited testing.

Processing tips

Pipetting

When pipetting the Buffer BL3 and isopropanol into the sample plate, we recommend pipetting down the side of the well instead of directly into the sample solution. Pipetting directly into solution can negatively affect the extraction performance and decrease the DNA purity.



Heterogeneity and viscosity

The extracted HMW DNA can be highly viscous and heterogeneous. This is normal and is one of the challenges of working with HMW DNA. The heterogeneity and viscosity of the DNA eluate will vary depending on sample type, DNA size, and sample input. Listed below are tips for working with HMW DNA.

Following elution of the HMW DNA:

Pipette-mix the extracted DNA 5-10X with a standard P200 pipette. Pipette-mixing will help to loosen and coax the viscous DNA into solution. Moderate amounts of pipette mixing will not significantly impact DNA length. Pipette-mixing is a standard part of our DNA elution process. For greater accuracy, the pipette-mixed DNA should be left overnight at room temperature before quantifying the concentration.

To accurately quantify the HMW DNA:

Pipette-mix the DNA 5X with a standard P200 pipette again. Perform a Nanodrop reading of the eluate to determine DNA purity. To accurately determine the concentration of dsDNA, we recommend using the Qubit dsDNA BR Assay.

In some cases, the extracted DNA will be very heterogeneous and contain large amounts of unsolubilized “jellies”:

If the extracted DNA is very heterogeneous or contains large amounts of unsolubilized “jellies”, we recommend triplicate measurements to ensure accurate quantification. If the concentration %CV exceeds 30% or if noticeable “jellies” remain, pipette-mix 10X with a standard P200 pipette or needle shear 5X with a 26g blunt end needle and allow DNA to rest at room temperature for 2 hours. Take care to disrupt any regions that feel more viscous than other regions. Re-quantify DNA sample.

If the extracted DNA needs to be used or quantified immediately after extraction:

The extracted DNA can be sheared 5X using a 26g blunt end stainless-steel needle and 1 mL syringe. Moderate amounts of needle shearing will not significantly impact DNA length. This will decrease heterogeneity, improving quantification accuracy.

Shearing HMW DNA

Concentrated, HMW DNA can be difficult to shear with either the Covaris g-TUBE or Diagenode Megaruptor 3 Hydropore - long. In some cases, the viscous DNA could clog the shearing consumables. For these samples, we recommend trying the following:

1. Pre-shear the HMW DNA 5X using a 26g blunt end stainless-steel needle and 1 mL syringe.
2. Pre-shear using the Megaruptor 3 DNAFluid+ Kit. We recommend 100 μ L of sample, concentrations <500 ng/ μ L, and a speed setting of 59.

Both options will help to decrease the sample viscosity without negatively affecting sequencing performance.

Following pre-shearing, follow the shearing guidelines outlined in the appropriate library prep [Procedure & checklist](#).

For high-throughput shearing solutions, please contact PacBio technical support.

Programs

As of the document release date, the following program scripts as well as the corresponding Procedures & checklists are available for HMW (50–300 kb) DNA extraction. They are recommended for most long-read sequencing applications. This includes PacBio HiFi sequencing.

Nanobind HT Protocol and Script Part Numbers					
Sample		KingFisher Apex	KingFisher Duo Prime	KingFisher Flex	Hamilton NIMBUS Presto
Cultured cells	Procedure & checklist	102-996-100	102-996-200	102-996-300	102-996-400
	Script	102-998-700	102-998-800	102-998-900	102-999-300
200 μ L mammalian whole blood	Procedure & checklist	102-995-700	102-995-800	102-995-900	102-996-000
	Script	102-998-400	102-998-500	102-998-600	102-999-300
1 mL mammalian whole blood	Procedure & checklist	102-995-300	102-995-400	102-995-500	102-995-200
	Script	102-998-100	102-998-200	102-998-300	102-999-000

Both the Nanobind HT CBB kit and Nanobind HT 1 mL blood kit support four 24 sample extractions runs (96 samples). The Nanobind HT kits are intended for automated extraction only. They are not compatible with manual extraction.

Please contact your Hamilton representative to install NIMBUS Presto scripts and contact PacBio (support@pacb.com) to obtain KingFisher Apex, Flex, or Duo Prime scripts.

Cultured cells – Nanobind HT CBB kit

This procedure describes the extraction of HMW DNA from cultured cells. It has been validated on several cell types including GM12878, GM24385, and MCF-7. They have been validated using fresh and frozen cell pellets. These protocols require the Nanobind HT CBB kit (102-762-700).

200 μ L mammalian whole blood – Nanobind HT CBB kit

This procedure describes the extraction of HMW DNA from 200 μ L of mammalian whole blood. It has been validated using fresh and frozen human whole blood. This protocol requires the Nanobind HT CBB kit (102-762-700).

1 mL mammalian whole blood – Nanobind HT 1 mL blood kit

This procedure describes the extraction of HMW DNA from 1 mL of mammalian whole blood. It has been validated using fresh and frozen human whole blood. This protocol requires the Nanobind HT 1 mL blood kit (102-762-800).

QC procedures

We recommend performing a Nanodrop UV/Vis measurement for DNA purity, a Qubit BR DNA Assay measurement for DNA yield, and an optional Qubit BR RNA Assay measurement. Additionally, we recommend Agilent Femto Pulse for DNA size QC.

See individual HMW DNA extraction protocols for detailed guidance.

Storage of DNA

DNA can be stored in Nanobind HT kit Buffer EB at 4°C for several months. Long term storage at -20°C or -80°C can be used if necessary. Avoid freeze-thaw cycles since this can degrade HMW DNA.

Troubleshooting FAQ

See individual DNA extraction protocols for details.

Revision history (description)	Version	Date
Initial release	01	April 2023

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