Protocol guide v1.0

Bead Xtract gDNA Kit

For Extraction of Genomic DNA from Blood and Saliva

Nonacus Bead Xtract gDNA Kit (96)
(Cat #PRE_EXT_BXG_96)

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ADVANCING NON-INVASIVE HEALTHCARE

Intended Use

This protocol is intended to provide guidance for using the Nonacus Bead Xtract gDNA kit (Catalogue No. PRE_EXT_BXG_96). Using the Nonacus Bead Xtract gDNA Kit, high quality genomic DNA can be extracted from blood and saliva samples and is suitable for any downstream applications, including qPCR and Next Generation Sequencing. The kit provides enough reagents to perform 96 extractions.

Key Features

- Fast and simple protocol, which enables extraction of genomic DNA in under 2 hours.
- Can be used for extraction from whole blood or saliva
- The Nonacus Bead Xtract gDNA Kit can be processed manually or using automated platforms
- The procedure eliminates the need for funnels and vacuum steps, providing hands-free operation in automated protocols
- The unique formulation of the lysis buffer and magnetic beads allow complete automation of the extraction process with minimal user intervention
- The high binding capacity of the beads allows for maximum recovery of DNA from the sample, with high concentration even when eluting in low volumes (50-100 µl).
- The system utilises the reversible nucleic acid-binding properties of paramagnetic particles to make processing DNA simple, clean and efficient
- Configurable elution volumes between 50-100 µL

Kit Contents

Nonacus Bead Xtract gDNA Kit for 96 extractions (Catalogue No. PRE_EXT_BXG_96)

Reagent	Quantity	Storage Conditions
gDNA Lysis Buffer	35 mL	15-25 °C
gDNA Wash Buffer 1	30 mL	15-25 °C
gDNA Wash Buffer 2	35 mL	15-25 °C
Elution Buffer	16 mL	15-25 °C
Proteinase K	46 mg	Pre-reconstitution store at 15-25°C Post-reconstitution store at -18 to -25°C
gDNA Magnetic Beads	3.5 mL	Refrigerated (2 to 8 °C)

Additional Required Materials/Equipment (Not supplied)

- 100% ethanol (molecular biology grade)
- 100% isopropanol (molecular biology grade)
- Nuclease-free water (molecular biology grade)
- Magnetic separation device for 1.5/2.0 ml microcentrifuge tubes (for all input volumes)
- Incubator or heat block capable of reaching and holding 70 °C temperature. If possible, a thermomixer is recommended to allow continuous vortexing whilst heating
- Vortex mixer
- 1.5/2.0 ml microcentrifuge tubes compatible with magnetic separation device used
- Optional: RNase A (10 mg/mL)
- Optional: 1X PBS (Phosphate Buffered Saline) for meeting total sample volume recommendations
- For quantification of extracted gDNA: Qubit™ dsDNA Broad Range Kit (ThermoFisher – Q32853)
- Optional: For qualification of extracted gDNA: Genomic DNA Screentape (Agilent Technologies - 5067-5365), and Genomic DNA Reagents (Agilent Technologies - 5067-5366). Tapestation 4200 system (Agilent Technologies - G2991BA) is required to run these

Storage and Stability

All Nonacus Bead Xtract gDNA Kit components are guaranteed for at least 6 months from the date of shipping when stored as follows:

- gDNA Magnetic Particles should be stored at 2 to 8°C for long-term use
- Lyophilised Proteinase K should be stored at 15-25°C for up to 12 months and must be stored at -18 to -25°C after initial reconstitution
- Store all other components at 15-25°C. During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve such deposits by warming the solution at 37 °C and gently shaking
- Do not use the Bead Xtract gDNA kit or any components past the expiry date stated

Considerations for Sample Types Used

Whole Blood
Whole blood must be collected in an EDTA tube. A volume of 200 µL is required for extraction. **DO NOT** use heparin tubes which can negatively impact the use of DNA in downstream applications.

Saliva
Saliva should be collected in stabilising solution following the manufacturer's instructions. A volume of 500 µL of saliva in stabilising solution is required for the extraction.

Reagent Preparation

Before using the kit for the first time, some of the reagents require additives. See table 1 below for details on how to prepare the reagents. Proteinase K is supplied in lyophilised form and needs to be reconstituted in nuclease free water before use.

Buffer	Diluent	Volume to add (mL)
gDNA Wash Buffer 1	100% Ethanol (molecular biology grade)	45
gDNA Wash Buffer 2	100% Ethanol (molecular biology grade)	105
Proteinase K	Nuclease-free water (molecular biology grade)	2.3

Table 1. Reagent dilutions required before starting. Add the listed amount of diluent to the entire bottle of reagent for use in all variations of the protocol

For long term storage, Proteinase K must be kept at -18 to -25°C after reconstitution and can be aliquoted for ease of use.

Nonacus Bead Xtract gDNA: Extraction from Whole Blood

Before starting:

1. Prepare buffers according to the reagent preparation instructions in Table 1 (page 4).
2. Ensure all reagents are equilibrated to room temperature, including Proteinase K and gDNA Magnetic Beads.
3. Set a heat block, water bath or thermomixer to 70°C.
4. Shake or vortex mix the gDNA Magnetic Beads to fully homogenise before use.

Protocol:

1. Vortex whole blood samples before use. Transfer 200 µL of the sample to a 1.5 mL or 2 mL microcentrifuge tube.
2. Prepare a fresh mastermix of Proteinase K and gDNA Lysis Buffer as instructed in the table below. If preparing a mastermix for more than 1 reaction, increase the amounts of the components by 5% to account for pipetting losses.

Component	Amount per sample (µL)	Amount for 8 samples +5% coverage (µL)
Proteinase K	20	168
gDNA Lysis Buffer	300	2520

⚠ **WARNING: Proteinase K - gNDA Lysis Buffer mastermix should be used within 2 hours**

3. Add 320 µL of Proteinase K – gDNA Lysis Buffer mastermix to each sample.
4. Mix by vortexing for 30 seconds and incubate at 70 °C for 10 minutes, with continuous mixing. If constant vortexing is not possible, perform a second vortex mix of 30 seconds after the first 5 minutes of incubation.

- **Optional:** Add 5 µL of RNase A, mix well by vortexing for 30 seconds and incubate for 2 minutes at room temperature.
- Once step 4 is complete, set the heat block, water bath or thermomixer to 60 °C (for use in step 19).

5. Prepare a fresh mastermix of 100% isopropanol and gDNA Magnetic Beads as instructed in the table below. If preparing a mastermix for more than 1 reaction, increase the amounts of the components by 5% to account for pipetting losses.

Component	Amount per sample (µL)	Amount for 8 samples +5% coverage (µL)
100% Isopropanol (molecular biology grade)	450	3780
gDNA Magnetic Beads	30	252

⚠ **WARNING: Proteinase K - gNDA Magentic Beads mastermix should be used within 2 hours**

6. Add 480 µL of Isopropanol– gDNA Magnetic Beads mastermix to each sample.

7. Mix by vortexing for 30 seconds and incubate at room temperature for 5 minutes, with continuous mixing. If constant vortexing is not possible, perform a second vortex mix of 30 seconds after the first 2 and a half minutes of incubation.

8. Place the tube on a magnetic separation device for at least 5 minutes.

9. Remove and discard the supernatant, retaining the beads.

10. Remove the tube from the magnetic separation device and add 700 µL of gDNA Wash Buffer 1.

11. Vortex mix for 30 seconds and place on the magnetic rack for at least 1 minute or until the beads clear from the solution.

12. Remove and discard the supernatant, retaining the beads.

13. Remove the tube from the magnetic separation device and add 700 µL of gDNA Wash Buffer 2.

14. Vortex mix for 30 seconds and place on the magnetic rack for at least 1 minute or until the beads clear from the solution.

15. Remove and discard the supernatant, retaining the beads.

16. Repeat steps 13-15 for a total of 2 washes with gDNA Wash Buffer 2.

17. Using a 10 µl pipette, remove any remaining supernatant and allow the beads to dry for 5 minutes at room temperature.

18. Add between 50 and 100 µL of gDNA Elution Buffer, depending on the desired final elution volume.

19. Vortex mix for 30 seconds and incubate at 60°C for 5 minutes.

20. Briefly centrifuge to collect the condensation from the cap and place the tube on the magnetic rack for 2 minutes or until the elution is clear of beads.

21. Avoiding the beads, transfer 50-100 µL eluate to a new tube for storage or downstream processing.

Nonacus Bead Xtract gDNA: Extraction from Stabilised Saliva

Before starting:

1. Prepare buffers according to the reagent preparation instructions in Table 1 (page 4).
2. Ensure all reagents are equilibrated to room temperature, including Proteinase K and gDNA Magnetic Beads.
3. Set a heat block, water bath or thermomixer to 70°C.
4. Shake or vortex mix the gDNA Magentic Beads to fully resuspend before use.

Protocol:

1. Thoroughly vortex the saliva sample to ensure that it is well mixed. Transfer 500 µL into a 2 mL microcentrifuge tube for extraction.
2. Add 20 µL of proteinase K to the sample. Mix by vortexing for 30 seconds and heat at 70°C for 10 minutes, with continuous mixing. If constant vortexing is not possible, perform a second vortex mix of 30 seconds after the first 5 minutes of incubation.

- **Optional:** Add 5 µL of RNase A, mix well by vortexing for 30 seconds and incubate for 2 minutes at room temperature.
- Once step 2 is complete, set the heat block, water bath or thermomixer to 60 °C (for use in step 17).

3. Prepare a fresh mastermix of 100% isopropanol and gDNA Magnetic Beads as instructed in the table below. If preparing a mastermix for more than 1 reaction, increase the amounts of the components by 5% to account for pipetting losses.

Component	Amount per sample (µL)	Amount for 8 samples +5% coverage (µL)
100% Isopropanol (molecular biology grade)	450	3780
gDNA Magnetic Beads	30	252

⚠ **WARNING: Proteinase K - gNDA Magentic Beads mastermix should be used within 2 hours**

4. Add 480 µL of Binding Buffer – gDNA Magnetic Beads mastermix to each sample.
5. Mix by vortexing for 30 seconds and incubate at Room Temperature for 5 minutes, with continuous mixing. If constant vortexing is not possible, perform a second vortex mix of 30 seconds after the first 2 and a half minutes of incubation.
6. Place the tube on a magnetic separation device for at least 5 minutes.
7. Remove and discard the supernatant, retaining the beads.
8. Remove the tube from the magnetic separation device and add 700 µL of gDNA Wash Buffer 1.
9. Vortex mix for 30 seconds and place on the magnetic rack for at least 1 minute or until the beads clear from the solution.
10. Remove and discard the supernatant, retaining the beads.
11. Remove the tube from the magnetic separation device and add 700 µL of gDNA Wash Buffer 2.

12. Vortex mix for 30 seconds and place on the magnetic rack for at least 1 minute or until the beads clear from the solution.

13. Remove and discard the supernatant, retaining the beads.

14. Repeat steps 11-13 for a total of 2 washes with gDNA Wash Buffer 2.

15. Using a 10 µl pipette, remove any remaining supernatant and allow the beads to dry for 5 minutes at room temperature.

16. Add 50-100 µL of Elution Buffer, depending on the desired final volume.

17. Vortex mix for 30 seconds and incubate at 60 °C for 5 minutes.

18. Briefly spin down to collect the condensation from the cap and place the tube on the magnetic rack for 2 minutes or until the elution is clear of beads.

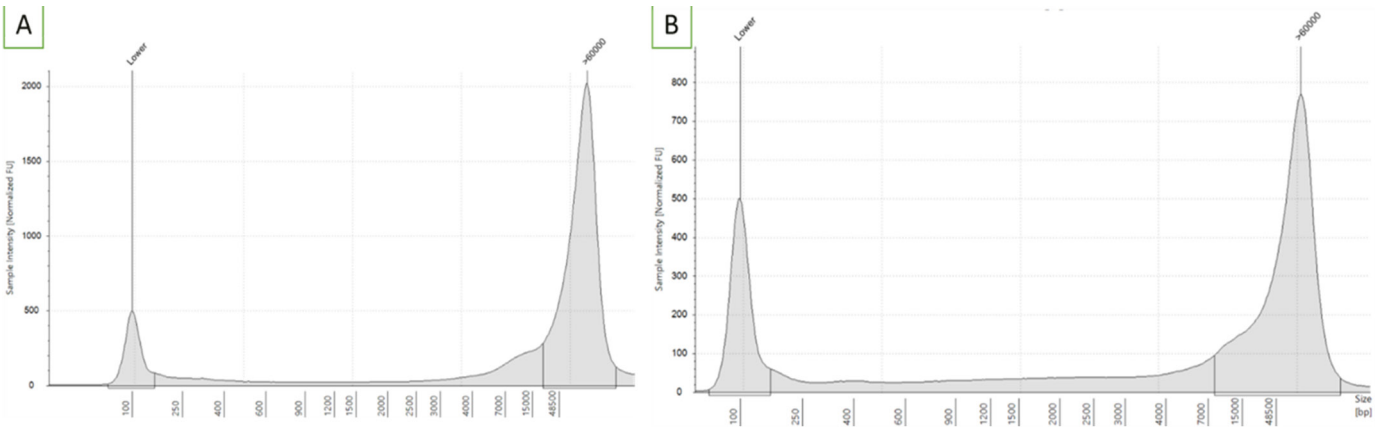
19. Avoiding the beads, transfer 50-100 µL eluate to a new tube for storage or downstream processing.

Quality Control Procedure for Extracted gDNA

Yields of genomic DNA obtained should be between 2-6 µg from blood or 6-13 µg from saliva if using the input amounts from this protocol. Yields will vary depending on input volume, biological sample variability and collection and storage conditions. A fluorometric assay such as the Qubit™ dsDNA Broad Range kit (Thermofisher-Q32853) can be used to quantify the gDNA yields extracted from blood and saliva. Use of UV-Vis spectrophotometry methods (e.g. Nanodrop) for quantifying nucleic acids is not recommended.

To check gDNA quality, a Tapestation 4200 (Agilent Technologies) instrument is recommended, using genomic DNA ScreenTape. Peaks on the electropherogram are anticipated to appear at >60,000 bp.

Figure 1. TapeStation electropherogram traces of genomic DNA extracted from blood (A) and saliva (B) (Saliva diluted 1/5) showing peak sizing >60,000 bp.



Automated Protocols

Automated protocols available upon request.

Support

Please speak to us if you require support by contacting support@nonacus.com

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