Protocol guide v1.0

Bead Xtract cfDNA

Cell Free DNA Extraction from Plasma and Serum

Bead Xtract cfDNA kit (20) – (PRE_EXT_BXC_20)
Bead Xtract cfDNA kit (200) – (PRE_EXT_BXC_200)
Bead Xtract cfDNA (800) – (PRE_EXT_BXC_800)

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Intended use

This protocol is intended to provide guidance using the Bead Xtract – 20 - 200 and - 800 kits. Using the Bead Xtract technology, high quality cell-free DNA can be extracted from plasma and serum samples and is suitable for downstream applications such as qPCR and Next Generation Sequencing. **The three kit sizes provide enough reagents to process the following number of manual reactions, dependent on sample input volume:**

Kit size	Number of extractions with 1 ml input	Number of extractions with 2 ml inpit	Number of extractions with 4 ml input
C3232SK (small)	20	10	Text
C3232MK (medium)	200	100	Text
c3232LK (large	800	400	Text

NOTE: DNA recovery will vary between 1-100 ng/ml from plasma and serum, due to biological variability

Key features

- Fast and simple protocol, which enables a 75-minute processing time with 35 minutes hands-on time, to extract circulating cell-free DNA from up to 10 ml of plasma or serum samples.
- The Bead Xtract cfDNA 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 and binding buffers allow complete automation of the extraction process with minimal user intervention.
- The high binding capacity of the beads allows for lower volume of magnetic particles needed, thus reducing the final elution volume required (minimum 30 µl).
- The system combines the reversible nucleic acid-binding properties of Bead Xtract paramagnetic particles with a unique binding system that targets smaller DNA fragments (150-400 bp) to maximise cfDNA yield.
- Does not require carrier RNA, allowing for accurate and reliable quantitation of extracted cfDNA for downstream applications, such as qPCR and Next Generation Sequencing.

WARNING: The protocol describes the workflow using 1 ml of sample input. If using sample input >1 ml, throughout the protocol the symbol † denotes a change in volume or incubation time which is referred to in Table 2. Take care to follow these where required.

Bead Xtract cfDNA kit (20) - (Catalogue No. PRE_EXT_BXC_20)

Reagent	Quantity	Storage conditions
Lysis buffer	1 x 1.5 ml	Room temperature
Binding buffer	1 x 25 ml	Room temperature
GITC buffer	1 x 22 ml	Room temperature
EtOH wash buffer	1 x 5 ml	Room temperature
Elution buffer	1 x 30 ml	Room temperature
Proteinase K solution	1 x 350 µl	Room temperature
Magnetic particles	1 x 110 µl	Refrigerated (2-8 °C)

Bead Xtract cfDNA kit (200) - (Catalogue No. PRE_EXT_BXC_200)

Reagent	Quantity	Storage conditions
Lysis buffer	1 x 20 ml	Room temperature
Binding buffer	1 x 225 ml	Room temperature
GITC buffer	2 x 110 ml	Room temperature
EtOH wash buffer	2 x 25 ml	Room temperature
Elution buffer	1 x 250 ml	Room temperature
Proteinase K solution	1 x 4 ml	Room temperature
Magnetic particles	1 x 1.1 ml	Refrigerated (2-8 °C)

Bead Xtract cfDNA kit (800) - (Catalogue No. PRE_EXT_BXC_800)

Reagent	Quantity	Storage conditions
Lysis buffer	1 x 80 ml	Room temperature
Binding buffer	4 x 220 ml	Room temperature
GITC buffer	4 x 220 ml	Room temperature
EtOH wash buffer	4 x 50 ml	Room temperature
Elution buffer	2 x 250 ml	Room temperature
Proteinase K solution	1 x 14 ml	Room temperature
Magnetic particles	1 x 4.4 ml	Refrigerated (2-8 °C)

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Additional required materials/equipment (not supplied)

- 100% ethanol (molecular biology grade)
- Magnetic separation device for 1.5/2.0 ml microcentrifuge tubes (for all input volumes)
- Magnetic separation device for 15 ml tubes (if using 2-4 ml input volumes)
- Magnetic separation device for 50 ml tubes (if using 5-10 ml input volumes)
- Incubator or heat block capable of reaching and holding 60 °C temperature
- Shaker or rocker
- Vortex mixer
- 15 and/or 50 ml tubes compatible with magnetic separation device used (if extracting >1 ml of sample)
- 1.5 ml microcentrifuge tubes compatible with magnetic separation device used
- Optional: microplate for DNA storage
- Optional for QC of extracted cfDNA: Qubit™ dsDNA High Sensitivity kit (ThermoFisher- Q32854) and Qubit™ 4 Fluorometer (ThermoFisher-Q33238)
- Optional for QC of extracted cfDNA: High Sensitivity D1000 Screentape (Agilent Technologies- 5067-5584) and High Sensitivity D1000 Reagents (Agilent Technologies- 5067-5585) **OR** cell-free DNA Screentape (Agilent Technologies- 5067-5630) and cell-free DNA Reagents (Agilent Technologies- 5067-5631). Tapestation 4200 system (Agilent Technologies- G2991BA) required to run these.

Storage and stability

All of the Bead Xtract cfDNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Magnetic Particles should be stored at 2-8 °C for long-term use. Proteinase K Solution can be stored at room temperature for up to 12 months. For longer term storage, store Proteinase K Solution at 2-8 °C. Store all other components at room temperature. 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.

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Plasma isolation

Please note blood samples received in EDTA tubes should be stored at 4 °C and processed within 24 hours of blood draw. Samples received in Nonacus Cell3™ Preserver tubes (C3009ML) should be stored at room temperature and processed within 21 days of blood draw. Samples received in other blood stabilising tubes should be stored at room temperature and processed according to manufacturer guidelines. The sample should not be processed if received in any other tube, or if clotted.

NOTE: All work should be carried out in a Class II biosafety cabinet using standard aseptic techniques to maintain sample sterility.

1. Centrifuge the blood sample at 2,000 g for 10 minutes using a swinging bucket rotor.

2. Collect the separated plasma using a 1,000 µl pipette ensuring that the buffy coat remains undisturbed.

3. Aliquot into 1.5 – 2 ml sterile 'PCR Clean' microcentrifuge tubes.

4. Centrifuge at maximum speed (minimum 10,000 g) for 10 minutes in a microcentrifuge.

NOTE: The second centrifugation step ensures that isolated plasma is free of cell debris, which can reduce qPCR efficiency and result in higher Ct values.

5. Taking care not to disturb the pellet retrieve the supernatant using a 1,000 µl pipette and aliquot into a fresh sterile 'PCR Clean' microcentrifuge 1.5-2 ml tube.

6. Plasma can be stored at -20 °C for long term storage. The sample is now ready for cell free DNA extraction.

NOTE: Frozen plasma can be thawed at room temperature or in a heat block/bath at 37 °C and used for cfDNA extraction.

Reagent preparation

1. Dilute EtOH Wash Buffer with 100% molecular biology grade ethanol as follows and store at room temperature.

Table 1. Volumes of 100% Ethanol to be added in the EtOH Wash Buffer for the different Bead Xtract cfDNA kits (20, 200 and 800 reactions)

Kit	100% Ethanol to be added
Bead Xtract cfDNA kit (20)	20 ml
Bead Xtract cfDNA kit (200)	100 ml per bottle
Bead Xtract cfDNA kit (800)	200 ml per bottle

2. Shake or vortex mix the Magentic Particles to fully resuspend the particles before use

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Bead Xtract cfDNA kit - Protocol for 1-4 ml serum/ plasma

Before starting:

1. Prepare EtOH Wash Buffer according to the 'Reagent Preparation' section on Page 5.
2. Set an incubator or heat block to 60 °C.
3. Shake or vortex mix the Magnetic Particles to fully resuspend the particles before use.

Protocol

All procedures should be carried out in a sterile environment such as a Class II biosafety cabinet. Sample inputs between 500 µl to 10 ml can be used, with adjustments to reagent volumes and incubation conditions. We recommend using 1, 2 or 4 ml as input; changes are described in the table below.

Table 2. Variations in the protocol based on sample input volumes (1, 2 and 4 ml).

Step	Step Description	Input volume:		
		1 ml	2 ml	4 ml
2	Proteinase K	15 µl	300 µl	60 µl
3	Lysis buffer	67 µl	135 µl	270 µl
5	60 °C incubation	20 minutes	25 minutes	30 minutes
7	Binding buffer	1 ml	2 ml	4 ml
8	Magentic particles	5 µl	10 µl	20 µl
16	GITC buffer	500 µl	1 ml	1 ml
20	GITC buffer	500 µl	1 ml	1 ml
22	EtOH wash buffer	500 µl	1 ml	1 ml
26	EtOH wash buffer	500	1 ml	1 ml

WARNING: The protocol describes the workflow using 1 ml of sample input. If using sample input >1 ml, throughout the protocol the symbol † denotes a change in volume or incubation time which is referred to in Table 2. Take care to follow these where required.

1. Add 500-1000 µl of serum/plasma to a 15 ml centrifuge tube. Bring the volume up to 1 ml with Elution Buffer if sample volume is less than 1 ml.

   NOTE: For sample input >1 ml, transfer plasma/serum to a 15 mL centrifuge tube and top up to 2mL or 4mL with elution buffer as required.

2. Add 15 µl † of Proteinase K Solution.
3. Add 67 µl † Lysis Buffer.

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4. Vortex mix at maximum speed or pipette mix thoroughly.

5. Incubate at 60 ºC for 20 minutes †. Mix by inverting or shaking every 10 minutes.

6. Incubate at room temperature for 10 minutes.

7. Add 1 ml † of Binding Buffer. Vortex mix at maximum speed for 30 seconds or pipette mix thoroughly.

8. Add 5 µl † of fully resuspended Magnetic Particles. Invert the sample 10 times or pipette to mix thoroughly. Incubate for 10 minutes at room temperature with continuous mixing. The samples must be mixed throughout the 10-minute incubation period by shaking, rocking or inverting. **Do not vortex mix at high speeds** as this will cause excess foaming that can reduce yield. The speed of mixing should be set to continuously keep the Magnetic Particles resuspended in solution.

9. Transfer 1 ml of lysate to a 1.5 ml microcentrifuge tube. Keep the remaining lysate for step 12 and do not discard.

   NOTE: For sample input >1 ml, skip steps 9-12. A magnet compatible with 15 ml tubes should be used for Steps 13 and 14.

10. Place the tube on the magnet and incubate at room temperature until the Magnetic Particles are completely cleared from solution.

11. With the tube still on the magnet, aspirate and discard the cleared supernatant. Do not disturb the Magnetic Particles.

12. Transfer the remaining lysate from Step 8 to the same 1.5 ml microcentrifuge tube used in the previous steps.

13. Place the tube on the magnet. Incubate at room temperature until the Magnetic Particles are completely cleared from solution.

14. With the tube still on the magnet, aspirate and discard the cleared supernatant. Do not disturb the Magnetic Particles.

15. Remove the tube containing the Magnetic Particles from the magnet.

16. Add 500 µl † of GITC Buffer.

17. Vortex mix for 2 minutes to resuspend the Magnetic Particles in GITC Buffer.

    NOTE: Complete resuspension of the Magnetic Particles is critical for obtaining good purity.

    NOTE: For sample input >1 ml, transfer the resuspended Magnetic Particles/GITC Buffer to a new 1.5 ml microcentrifuge tube. Use a magnet designed for 1.5/2.0 ml tubes for the remaining procedure.

18. Place the tube on the magnet. Incubate at room temperature until the Magnetic Particles are completely cleared from solution.

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19. With the tube still on the magnet, aspirate and discard the cleared supernatant. Do not disturb the Magnetic Particles.

20. Repeat Steps 15-19 for a second GITC Buffer step.

21. Remove the tube containing the Magnetic Particles from the magnet.

22. Add 500 µl † of EtOH Wash Buffer.

NOTE: EtOH Wash Buffer must be diluted with 100% ethanol prior to use (see **Table 1** for instructions).

23. Vortex mix for 2 minutes to resuspend the Magnetic Particles in EtOH Wash Buffer.

24. Place the tube on the magnet. Incubate at room temperature until the Magnetic Particles are completely cleared from solution.

25. With the tube still on the magnet, aspirate and discard the cleared supernatant. Do not disturb the Magnetic Particles.

26. Repeat Steps 21-25 for a second EtOH Wash Buffer wash step.

27. Remove the tube from the magnet for approximately 30 seconds.

28. Place the tube on the magnet. Incubate at room temperature until the Magnetic Particles are completely cleared from solution.

29. With the tube still on the magnet, aspirate and discard the residual EtOH Wash Buffer using a 10 µl pipette.

30. Leave the tube on the magnet with the lid open for 25 minutes to dry the Magnetic Particles.

31. Remove the tube containing the Magnetic Particles from the magnet.

32. Add 30-60 µl † of Elution Buffer.

33. Vortex mix at room temperature for 5 minutes to resuspend the Magnetic Particles in Elution Buffer.

34. Place the tube on the magnet. Incubate at room temperature until the Magnetic Particles are completely cleared from solution.

35. With the tube still on the magnet, transfer the cleared supernatant containing purified cfDNA to a PCR-clean 1.5 ml microcentrifuge tube or microplate, taking extra care to avoid disturbing the bead pellet (carryover of magnetic particles into the final eluate may negatively impact downstream applications).

36. The extracted cfDNA is ready for use or can be stored at -20 °C for long term storage.

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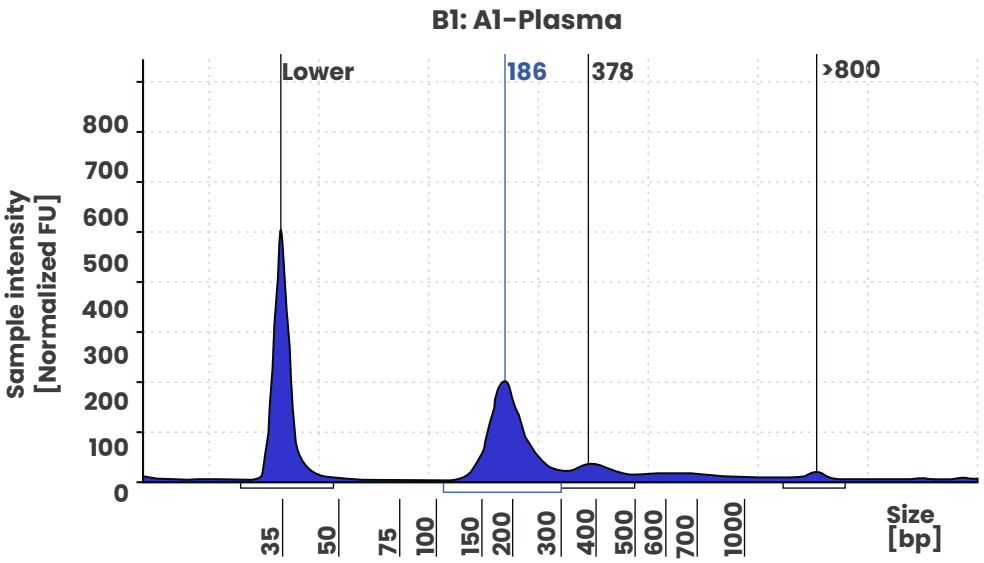
Quality control procedure for extracted cfDNA

Yields of cell-free DNA will vary between 1-100 ng/ml from plasma and serum, depending on input amount and biological sample variability. A fluorometric assay such as the Qubit™ dsDNA High Sensitivity (ThermoFisher-Q32854) kit can be used to quantify cfDNA.

To check cfDNA fragment size, Tapestation 4200 (Agilent Technologies) is recommended, using either a D1000 high sensitivity screentape or a cfDNA screentape. The profile for cfDNA should look like the below:



Depending on screentape assay used, the main mono-nucleosome peak should fall at approximately 180 bp and the following multimer peaks will be multiples of this.

Automated Protocols

Automated methods for Bead Xtract cfDNA are available from Nonacus for the following:

- Thermofisher KingFisher Duo/Duo Prime and KingFisher Flex (2 ml and 4 ml input)
- Hamilton STAR (4 ml input), channels only and 96-head versions

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

Nonacus Limited
Quinton Business Park
11 Ridgeway
Birmingham
B32 1AF