STAR BEADS cfDNA/RNA EXTRACTION Kit

NB-78-00019-1x10
NB-78-00019-1x96

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1.General information

Description

STAR BEADS cfDNA/RNA Extraction Kit provides a fast and efficient purification method to isolate high-quality cell free DNA and RNA from body fluids such as human EDTA plasma, serum and cerebrospinal fluid for reliable downstream applications.

The STAR BEADS Kit is based on magnetic bead technology and it can be used for rapid manual extraction and also for automated extraction on automatic magnetic separators, with the dedicated extraction protocol. The procedure's time depends on the instrument's configuration and the magnetic separation system used. The amount of recovered cell free DNA /RNA depends on the type of sample and pre-analytical sample handling.

2. Principle

The procedure is based on the reversible adsorption of nucleic acids to STAR BEADS Magnetic Beads in appropriate buffers, while impurities are efficiently removed during the washing steps.

Lysis of the sample is performed in STAR BEADS Lysis Buffer 2 with the addition of Proteinase K. Binding of nucleic acids to STAR BEADS Magnetic Beads is performed in STAR BEADS Binding Buffer 2. After magnetic separation, the magnetic beads are washed with two washing reagents (STAR BEADS Washing Buffer 1G and STAR BEADS Washing Buffer 2G) to remove contaminants and salts. Finally, purified cfDNA/RNA is eluted with STAR BEADS Elution Buffer 1 which causes the nucleic acid to detach from the magnetic beads. The resulting high-quality cfDNA/RNA is then ready for use in downstream applications such as RT-PCR, qRT-PCR, sequencing, or any other type of enzymatic reaction, or it can be frozen for later use.

1.3 Intended use

STAR BEADS cfDNA/RNA Extraction Kit is intended for use for the extraction of high-quality cfDNA and RNA from body fluids such as human EDTA plasma, serum and cerebrospinal fluid. The kit is intended for “Research Use Only” (RUO).

The STAR BEADS cfDNA/RNA Extraction Kit is intended to be used at a temperature between +15°C and 25°C. Use outside of this temperature range may result in suboptimal results.

1.4 Limitations of Use

The STAR BEADS cfDNA/RNA Extraction Kit is only intended for use with plasma prepared from human whole blood samples collected in EDTA tubes. It is not intended for use with samples stored in other collection tubes.

The STAR BEADS cfDNA/RNA Extraction Kit is not intended for use in diagnostic procedures.

The product has not been tested for drug development and it is unsuitable for administration to humans or animals.

1.5 Kit specifications

The STAR BEADS cfDNA/RNA Extraction Kit is recommended for the rapid manual and automated isolation of circulating cell-free DNA and RNA from human EDTA plasma, serum and cerebrospinal fluid. The cell free DNA/RNA yield strongly depends on the individual sample, storage and quality. Elution can be performed in the range 30–200 µL of elution buffer, and the eluted cfDNA/RNA is ready to use for subsequent downstream applications. For applications requiring more concentrated cfDNA/RNA the elution volume can be scaled down to 30 µL.

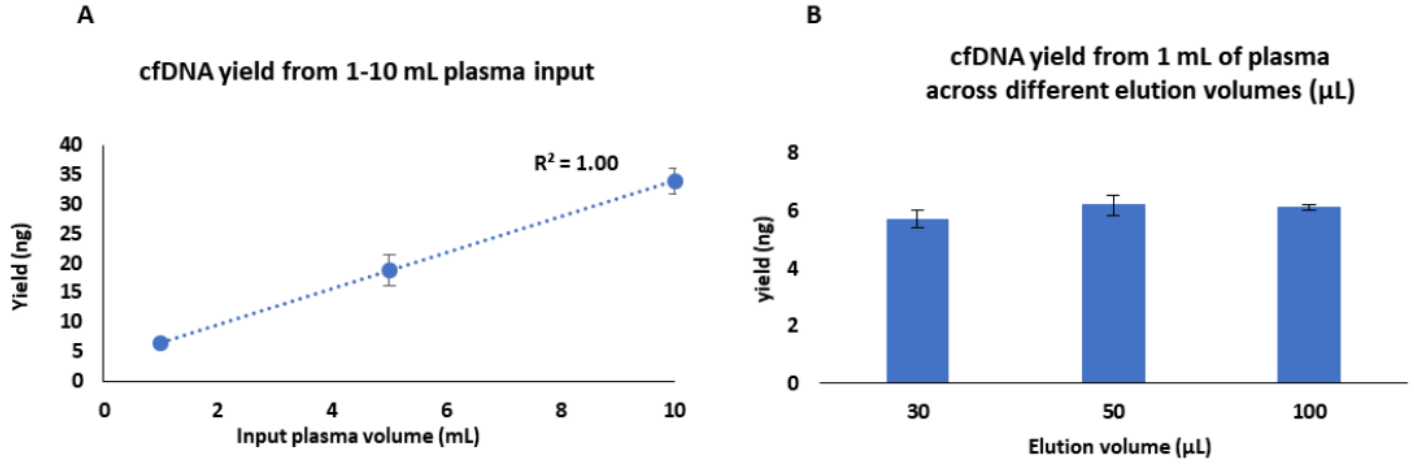
Parameter	STAR BEADS cfDNA/RNA Extraction Kit
Technology	Magnetic bead technology
Sample material	Human EDTA plasma, serum, cerebrospinal fluid
Sample amount	1-10 mL
Typical yield	Depending on sample source, storage and quality
Elution volume	30-200µL

Kit specifications

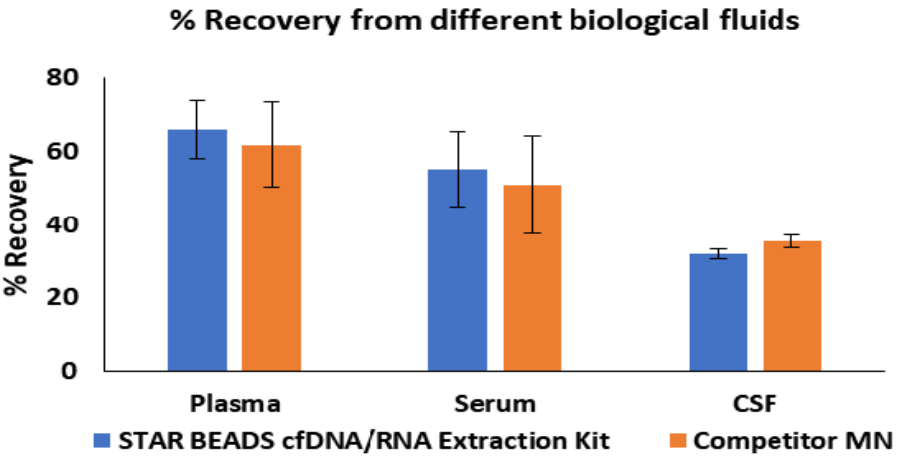
The STAR BEADS cfDNA/RNA Extraction Kit is designed to extract cfDNA and RNA from 1 mL of biological fluid, but it can also be scaled individually up to 10 mL. In the table below are described the estimated number of extractions according to the different sample volumes:

Sample volume (mL)	Number of extractions NB-78-00019-1x96
1	96
2	48
5	20
10	10

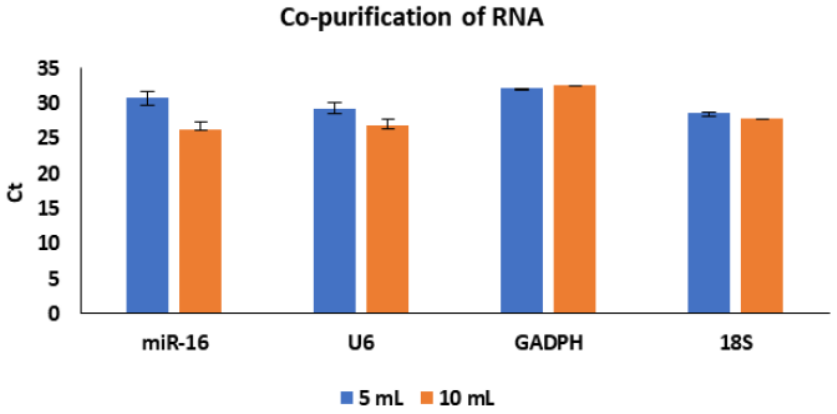
To adapt the volume of each STAR BEADS buffers to the sample volume please refer to section 5.



Flexible performance across a range of sample inputs and elution volumes. cfDNA was isolated using STAR BEADS cfDNA/RNA Extraction Kit from plasma from healthy donor. Yield was determined by fluorescent dye quantitation (Green Quant dsDNA) on Victor NIVO (Revvity).
Panel A. The cfDNA yield scales linearly across 1–10 ml of plasma volume inputs. Data represent mean ±SD, N=4 for 1 mL input; N=6 for 5 mL input and N=4 for 10 mL input.
Panel B. Yield of cfDNA is consistent between 30µl and 100µl of elution buffer volumes. Data represent mean ±SD, N=2.



Compatibility with different biological fluids. Percent recovery of a DNA ladder in the range of 25 bp to 700 bp (Generuler Low range DNA ladder, Thermo Scientific) spiked into 1 mL DNA Cleared Plasma (SBI), human serum (Sigma Aldrich) and Artificial Cerebrospinal fluid (Tocris) at a concentration of 100 ng/mL. DNA was extracted using STAR BEADS cfDNA/RNA Extraction Kit and Competitor MN according to the manufacturer's instructions. Isolated DNA was quantified with GreenQuant dsDNA Kit on Victor Nivo (Revvity). Data represent mean ±SD, N=3.



Co-purification of cell free RNA from the same sample. Realtime RT-qPCR of miR-16 (miRNA), U6 (snRNA), GADPH (mRNA) and 18S (rRNA) from eluates obtained from 5 and 10 mL of EDTA-plasma using STAR BEADS cfDNA/RNA Extraction Kit. For miR-16 and U6, 9 µL of eluate were used in a total volume of 15 µL for cDNA synthesis and then 4 µL were used for qPCR. For GADPH and 18S, 5 µL of eluate were directly amplified in one step qPCR. Data expressed in Ct mean ± SD, N=4 for mir-16 and U6; N=2 for GADPH and 18S.

2. Components, shipping and storage conditions and other required materials

2.1 Kit content

The Kit is available in bottle format.

Bottle format: NB-78-00019-1x10 , NB-78-00019-1x96. Use for manual procedure or in combination with Allsheng Auto-Pure 24, MOLGEN PurePrep 24 and liquid handling systems.

Components	GHS	NB-78-00019-1x10 (10preps)	NB-78-00019-1x96 (96 preps)
STAR BEADS Lysis Buffer 2 *		5 mL	50 mL
STAR BEADS Binding Buffer 2		12 mL	100 mL
STAR BEADS Magnetic Beads		1 mL	7 mL
STAR BEADS Washing Buffer 1G*		12 mL	100 mL
			
STAR BEADS Washing Buffer 2G		25 mL	200 mL
STAR BEADS Elution Buffer 1	None	1.5 mL	15 mL
STAR BEADS Proteinase K		1x 0.750 mL	4x 0.750 mL

* Contains chaotropic salt. Take appropriate laboratory safety measures, and wear gloves when handling. Not compatible with disinfecting agents that contain bleach. See Safety Data Sheet for safety information.
Note: Please note that components from different batches cannot be used interchangeably.

2.2 Shipping and storage

The kit is to be used in combination with dedicated equipment and plastic consumables. See in section 2.4
The kit and all its components are shipped and must be stored at room temperature (RT) (+15 to 25°C). Do not use the product after the expiry date indicated on the label.

2.3 Eluates stability

For short-term storage, it is recommended to store at -20°C. For long-term storage, it is recommended to store at -80°C.

2.4 Required materials to be supplied by the user

- Micropipettes suitable for pipetting 10-20 µL, 150 µL, 300 µL, 500 µL;
- Disposable tips with DNAse / RNAse (filter tips recommended);
- Refrigerator at +4°C or low/very low temperature freezer at -20/
- -80°C for the storage of samples;
  Biological hood suitable for working with hazardous, infectious or biologically contaminated materials. Follow local guidelines for working in a safe and acceptable manner;
- Magnetic separation plate or magnet for separating magnetic beads;
- Benchtop vortex mixer;
- Water bath or heating block for plasma digestion;
- DNAse / RNAse-free tubes or plates;
- Plastic consumables (24-deep well plates and tip combs) for automated extraction.

3. Collection, handling and storage of sample material

For plasma preparation, handling and storage, refer to ISO 20186-3: “Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for venous whole blood - Part 3: Isolated circulating cell free DNA from plasma”.
For serum and cerebrospinal fluid handling and storage it is recommended to follow the same guidelines adopted for plasma samples.

4. Reagents preparation

Please take a few moments to read this handbook carefully before beginning your preparation.

4.1 STAR BEADS Lysis Buffer 2, STAR BEADS Binding Buffer 2, STAR BEADS Washing Buffer 1G

STAR BEADS Lysis Buffer 2 may form salt precipitates upon storage below +20-25°C. If any precipitate formed, incubate the buffer bottle at +40 °C until all of the precipitates is re-dissolved.

4.2 STAR BEADS Magnetic Beads

Before distributing the beads, make sure that the beads are completely re-suspended. Shake the storage bottle well or place it on a vortex shortly.
Magnetic separation time depends on the magnetic strength of the magnetic separator, on the distance of the separation plate from the magnetic pins, and on the volume to be processed. Optimization may be required for each system.

5. Protocol for the isolation of cfDNA/RNA (manual procedure)

Optimized procedures are available for isolation of cell-free DNA and RNA from a range of 1–10 mL of sample simply by adjusting each reagent used for the extraction as summarized in the table below:

Sample volume (mL)	STAR BEADS Proteinase K (µL)	STAR BEADS Lysis Buffer 2 (mL)	STAR BEADS Binding Buffer 2 (mL)	STAR BEADS Magnetic Beads (µL)	STAR BEADS Washing Buffer 1G/2G (mL)	STAR BEADS Elution Buffer 1 (µL)
1	25	0.45	1	60	1	30-100
2	50	0.9	2	120	1	50-100
5	125	2.25	5	240	2	50-200
10	250	4.5	10	360	2	50-200

5.1 Protocol for 1 mL of sample

Lyse the sample

Transfer in a clean DNAse/RNAse free 15 mL tube 1 mL of sample and 25 µL of **STAR BEADS Proteinase K**.
Mix carefully using a vortex and incubate at room temperature for 15 minutes.
Add 0.45 mL **STAR BEADS Lysis Buffer 2**and mix vigorously by vortexing.
Incubate at +56 °C for 30 min ideally with shaking.
When incubation is finished, allow the samples to cool down for 5 min.

Bind the nucleic acid

Add 1 mL of **STAR BEADS Binding Buffer 2** and 60 µL of **STAR BEADS Magnetic Beads** to the lysate sample.
Mix for 10 min at room temperature.
IMPORTANT: Make sure to vigorously shake to ensure optimal cfDNA/RNA binding to the beads. Insufficient shaking will result in lower cfDNA/RNA recovery yield.
Briefly spin down with a centrifuge.
Place the vials on a magnetic device at room temperature for 5 minutes or until the beads are completely cleared from solution.
Aspirate and discard supernatant without disturbing the bead pellet bound to cfDNA/RNA.

Wash magnetic beads (Wash 1)

Add 1 mL **STAR BEADS Washing Buffer 1G** to the bead-cfDNA/RNA pellet, and mix well by vortexing.
Transfer the bead-cfDNA/RNA suspension to a new clean tube.
Optional: To increase the recovery yield of cfDNA/RNA, do not discard the old tube and add into it 0,5 mL of **STAR BEADS Washing Buffer 1G** and rinse the walls of the tube pipetting up and down to be sure to recover residual beads and transfer into the new tube into which the bead-cfDNA/RNA pellet was previously collected.
Spin down with a centrifuge.
Place the vials on a magnetic device at room temperature for 5 minutes or until the beads are completely cleared from solution.
Remove the supernatant without disturbing the bead pellet bound to cfDNA/RNA.
Optional: To increase the recovery yield of cfDNA/RNA, do not discard the supernatant and see how process it in next Wash 2 step.

Wash magnetic beads (Wash 2)

Add 1 mL **STAR BEADS Washing Buffer 2G** to bead-cfDNA/RNA pellet and mix well by vortexing.
Optional: To increase the recovery yield of cfDNA/RNA, do not discard the supernatant from Wash 1 step, place the recovered supernatant on the magnetic device at room temperature for additional 2 minutes until the beads are completely cleared from solution. At this point, remove the supernatant, add 0.5 mL of **STAR BEADS Washing Buffer 2G**, mix well by vortexing and collect together with the bead-cfDNA/RNA suspension previously

collected in 1 mL of **STAR BEADS Washing Buffer 2G.**
Mix well by vortexing the bead-cfDNA/RNA suspension.
Spin down with a centrifuge.
Place the vials on a magnetic device at room temperature for 2 minutes or until the beads are completely cleared from solution.
Remove the supernatant without disturbing bead pellet bound to cfDNA/RNA.

Wash magnetic beads (Wash 2)

Add 1 mL **STAR BEADS Washing Buffer 2G** to bead-cfDNA/RNA pellet and mix well by vortexing.
Spin down with a centrifuge.
Place the vials on a magnetic device at room temperature for 2 minutes or until the beads are completely cleared from solution.
Remove the supernatant without disturbing bead pellet bound to cfDNA/RNA.

Dry magnetic beads

Incubate at room temperature for 5-10 min until the bead-cfDNA/RNA pellets are dried, avoiding excessive drying.

Elute highly pure cfDNA /RNA

Add **30-100 µL STAR BEADS Elution Buffer 1** and mix well by vortexing. Do not use the pipette to mix the magnetic beads. It is essential to cover the magnetic beads completely with elution buffer during this step.
Incubate at room temperature for 5 minutes and mix by vortexing several times.

Collect cfDNA /RNA

Place the vials on a magnetic device at room temperature for 5 minutes or until the beads are completely cleared from solution.
Collect the supernatant with cfDNA/RNA without disturbing the bead pellet and transfer it into a new DNAse/RNAse free tube.

Note:

In some cases, traces of STAR BEADS Magnetic Beads may be left in the eluate. An additional separation step using a magnetic separator is recommended to separate any traces of particles.

5.2 Protocol for 2 mL of sample

Lyse the sample

Transfer in a clean DNAse/RNAse free 15 mL tube 2 mL of sample and 50 µL of **STAR BEADS Proteinase K**.
Mix carefully using a vortex and incubate at room temperature for 15 minutes.
Add 0.9 mL **STAR BEADS Lysis Buffer 2**and mix vigorously by vortexing.
Incubate at +56 °C for 30 min ideally with shaking.
When incubation is finished, allow the samples to cool down for 5 min.

Bind the nucleic acid

Add 2 mL of **STAR BEADS Binding Buffer 2** and 120 µL of **STAR BEADS Magnetic Beads** to the lysate sample.
Mix for 10 min at room temperature.
IMPORTANT: Make sure to vigorously shake to ensure optimal cfDNA/RNA binding to the beads. Insufficient shaking will result in lower cfDNA/RNA recovery yield.
Briefly spin down with a centrifuge.
Place the vials on a magnetic device at room temperature for 5 minutes or until the beads are completely cleared from solution.
Aspirate and discard supernatant without disturbing the bead pellet bound to cfDNA/RNA.

Wash magnetic beads (Wash 1)
Add 1 mL **STAR BEADS Washing Buffer 1G** to the bead-cfDNA/RNA pellet, and mix well by vortexing.
Transfer the bead-cfDNA/RNA suspension to a new clean tube.
Optional: To increase the recovery yield of cfDNA/RNA, do not discard the old tube and add into it 0,5 mL of **STAR BEADS Washing Buffer 1G** and rinse the walls of the tube pipetting up and down to be sure to recover residual beads and transfer into the new tube into which the bead-cfDNA/RNA pellet was previously collected.
Spin down with a centrifuge.
Place the vials on a magnetic device at room temperature for 5 minutes or until the beads are completely cleared from solution.
Remove the supernatant without disturbing the bead pellet bound to cfDNA/RNA.
Optional: To increase the recovery yield of cfDNA/RNA, do not discard the supernatant and see how process it in next Wash 2 step.

Wash magnetic beads (Wash 2)
Add 1 mL **STAR BEADS Washing Buffer 2G** to bead-cfDNA/RNA pellet and mix well by vortexing.
Optional: To increase the recovery yield of cfDNA/RNA, do not discard the supernatant from Wash 1 step, place the recovered supernatant on the magnetic device at room temperature for additional 2 minutes until the beads are completely cleared from solution. At this point, remove the supernatant, add 0.5 mL of **STAR BEADS Washing Buffer 2G**, mix well by vortexing and collect together with the bead-cfDNA/RNA suspension previously collected in 1 mL of **STAR BEADS Washing Buffer 2G.**
Mix well by vortexing the bead-cfDNA/RNA suspension.
Spin down with a centrifuge.
Place the vials on a magnetic device at room temperature for 2 minutes or until the beads are completely cleared from solution.
Remove the supernatant without disturbing bead pellet bound to cfDNA/RNA.

Wash magnetic beads (Wash 2)
Add 1 mL **STAR BEADS Washing Buffer 2G** to bead-cfDNA/RNA pellet and mix well by vortexing.
Spin down with a centrifuge.
Place the vials on a magnetic device at room temperature for 2 minutes or until the beads are completely cleared from solution.
Remove the supernatant without disturbing bead pellet bound to cfDNA/RNA.

Dry magnetic beads
Incubate at room temperature for 5-10 min until the bead-cfDNA/RNA pellets are dried, avoiding excessive drying.

Elute highly pure cfDNA /RNA
Add **30-100 µL STAR BEADS Elution Buffer 1** and mix well by vortexing. Do not use the pipette to mix the magnetic beads. It is essential to cover the magnetic beads completely with elution buffer during this step.
Incubate at room temperature for 5 minutes and mix by vortexing several times.
Collect cfDNA /RNA
Place the vials on a magnetic device at room temperature for 5 minutes or until the beads are completely cleared from solution.
Collect the supernatant with cfDNA/RNA without disturbing the bead pellet and transfer it into a new DNAse/RNAse free tube.

Note:

In some cases, traces of STAR BEADS Magnetic Beads may be left in the eluate. An additional separation step using a magnetic separator is recommended to separate any traces of particles.

5.3 Protocol for 5 mL of sample

Lyse the sample

Transfer in a clean DNAse/RNAse free 15 mL tube 5 mL of sample and 125 µL of **STAR BEADS Proteinase K**.
Mix carefully using a vortex and incubate at room temperature for 15 minutes.
Add 2.25 mL **STAR BEADS Lysis Buffer 2**and mix vigorously by vortexing.
Incubate at +56 °C for 30 min ideally with shaking.
When incubation is finished, allow the samples to cool down for 5 min.

Bind the nucleic acid

Add 5 mL of **STAR BEADS Binding Buffer 2** and 240 µL of **STAR BEADS Magnetic Beads** to the lysate sample.
Mix for 10 min at room temperature.
IMPORTANT: Make sure to vigorously shake to ensure optimal cfDNA/RNA binding to the beads. Insufficient shaking will result in lower cfDNA/RNA recovery yield.
Briefly spin down with a centrifuge.
Place the vials on a magnetic device at room temperature for 5 minutes or until the beads are completely cleared from solution.
Aspirate and discard supernatant without disturbing the bead pellet bound to cfDNA/RNA.

Wash magnetic beads (Wash 1)

Add 2 mL **STAR BEADS Washing Buffer 1G** to the bead-cfDNA/RNA pellet, and mix well by vortexing.
Transfer the bead-cfDNA/RNA suspension to a new clean tube.
Optional: To increase the recovery yield of cfDNA/RNA, do not discard the old tube and add into it 0,5 mL of **STAR BEADS Washing Buffer 1G** and rinse the walls of the tube pipetting up and down to be sure to recover residual beads and transfer into the new tube into which the bead-cfDNA/RNA pellet was previously collected.
Spin down with a centrifuge.
Place the vials on a magnetic device at room temperature for 5 minutes or until the beads are completely cleared from solution.
Remove the supernatant without disturbing the bead pellet bound to cfDNA/RNA.

NOTE: To increase the recovery yield of cfDNA/RNA, do not discard the supernatant and see how process it in next Wash 2 step.

Wash magnetic beads (Wash 2)

Add 2 mL **STAR BEADS Washing Buffer 2G** to bead-cfDNA/RNA pellet and mix well by vortexing.
Optional: To increase the recovery yield of cfDNA/RNA, do not discard the supernatant from Wash 1 step, place the recovered supernatant on the magnetic device at room temperature for additional 2 minutes until the beads are completely cleared from solution. At this point, remove the supernatant, add 0.5 mL of **STAR BEADS Washing Buffer 2G**, mix well by vortexing and collect together with the bead-cfDNA/RNA suspension previously collected in 2 mL of **STAR BEADS Washing Buffer 2G.**
Mix well by vortexing the bead-cfDNA/RNA suspension.
Spin down with a centrifuge.
Place the vials on a magnetic device at room temperature for 2 minutes or until the beads are completely cleared from solution.
Remove the supernatant without disturbing bead pellet bound to cfDNA/RNA.

Wash magnetic beads (Wash 2)
Add 2 mL **STAR BEADS Washing Buffer 2G** to bead-cfDNA/RNA pellet and mix well by vortexing.
Spin down with a centrifuge.
Place the vials on a magnetic device at room temperature for 2 minutes or until the beads are completely cleared from solution.
Remove the supernatant without disturbing bead pellet bound to cfDNA/RNA.

Dry magnetic beads
Incubate at room temperature for 5-10 min until the bead-cfDNA/RNA pellets are dried, avoiding excessive drying.

Elute highly pure cfDNA /RNA
Add **50-200 µL STAR BEADS Elution Buffer 1** and mix well by vortexing. Do not use the pipette to mix the magnetic beads. It is essential to cover the magnetic beads completely with elution buffer during this step.
Incubate at room temperature for 5 minutes and mix by vortexing several times.

Collect cfDNA /RNA
Place the vials on a magnetic device at room temperature for 5 minutes or until the beads are completely cleared from solution.
Collect the supernatant with cfDNA/RNA without disturbing the bead pellet and transfer it into a new DNAse/RNAse free tube.

Note:
In some cases, traces of STAR BEADS Magnetic Beads may be left in the eluate. An additional separation step using a magnetic separator is recommended to separate any traces of particles.

5.4 Protocol for 10 mL of sample
Lyse the sample
Transfer in a clean DNAse/RNAse free 50 mL tube 10 mL of sample and 250 µL of **STAR BEADS Proteinase K**.
Mix carefully using a vortex and incubate at room temperature for 15 minutes.
Add 4.5 mL **STAR BEADS Lysis Buffer 2**and mix vigorously by vortexing.
Incubate at +56 °C for 30 min ideally with shaking.
When incubation is finished, allow the samples to cool down for 5 min.

Bind the nucleic acid
Add 10 mL of **STAR BEADS Binding Buffer 2** and 360 µL of **STAR BEADS Magnetic Beads** to the lysate sample.
Mix for 10 min at room temperature.
IMPORTANT: Make sure to vigorously shake to ensure optimal cfDNA/RNA binding to the beads. Insufficient shaking will result in lower cfDNA/RNA recovery yield.
Briefly spin down with a centrifuge.
Place the vials on a magnetic device at room temperature for 5 minutes or until the beads are completely cleared from solution.
Aspirate and discard supernatant without disturbing the bead pellet bound to cfDNA/RNA.

Wash magnetic beads (Wash 1)
Add 2 mL **STAR BEADS Washing Buffer 1G** to the bead-cfDNA/RNA pellet, and mix well by vortexing.
Transfer the bead-cfDNA/RNA suspension to a new clean tube.

Optional: To increase the recovery yield of cfDNA/RNA, do not discard the old tube and add into it 0,5 mL of **STAR BEADS Washing Buffer 1G** and rinse the walls of the tube pipetting up and down to be sure to recover residual beads and transfer into the new tube into which the bead-cfDNA/RNA pellet was previously collected.
Spin down with a centrifuge.
Place the vials on a magnetic device at room temperature for 5 minutes or until the beads are completely cleared from solution.
Remove the supernatant without disturbing the bead pellet bound to cfDNA/RNA.
Optional: To increase the recovery yield of cfDNA/RNA, do not discard the supernatant and see how process it in next Wash 2 step.

Wash magnetic beads (Wash 2)
Add 2 mL **STAR BEADS Washing Buffer 2G** to bead-cfDNA/RNA pellet and mix well by vortexing.
Optional: To increase the recovery yield of cfDNA/RNA, do not discard the supernatant from Wash 1 step, place the recovered supernatant on the magnetic device at room temperature for additional 2 minutes until the beads are completely cleared from solution. At this point, remove the supernatant, add 0.5 mL of **STAR BEADS Washing Buffer 2G**, mix well by vortexing and collect together with the bead-cfDNA/RNA suspension previously collected in 2 mL of **STAR BEADS Washing Buffer 2G.**
Mix well by vortexing the bead-cfDNA/RNA suspension.
Spin down with a centrifuge.
Place the vials on a magnetic device at room temperature for 2 minutes or until the beads are completely cleared from solution.
Remove the supernatant without disturbing bead pellet bound to cfDNA/RNA.

Wash magnetic beads (Wash 2)
Add 2 mL **STAR BEADS Washing Buffer 2G** to bead-cfDNA/RNA pellet and mix well by vortexing.
Spin down with a centrifuge.
Place the vials on a magnetic device at room temperature for 2 minutes or until the beads are completely cleared from solution.
Remove the supernatant without disturbing bead pellet bound to cfDNA/RNA.

Dry magnetic beads
Incubate at room temperature for 5-10 min until the bead-cfDNA/RNA pellets are dried, avoiding excessive drying.

Elute highly pure cfDNA /RNA
Add **50-200 µL STAR BEADS Elution Buffer 1** and mix well by vortexing. Do not use the pipette to mix the magnetic beads. It is essential to cover the magnetic beads completely with elution buffer during this step.
Incubate at room temperature for 5 minutes and mix by vortexing several times.

Collect cfDNA /RNA
Place the vials on a magnetic device at room temperature for 5 minutes or until the beads are completely cleared from solution.
Collect the supernatant with cfDNA/RNA without disturbing the bead pellet and transfer it into a new DNAse/RNAse free tube.

Note:

In some cases, traces of STAR BEADS Magnetic Beads may be left in the eluate. An additional separation step using a magnetic separator is recommended to separate any traces of particles.

6. Protocol for the isolation of cfDNA/RNA (automated procedure)

Automated extraction with STAR BEADS cfDNA/RNA Extraction Kit, bottle format REF NB-78-00019-1x10,1X10 - NB-78-00019-1x96 can be performed in combination with Auto-Pure 24 and MOLGEN PurePrep 24 and other liquid handling systems.

For protocol, script, customized protocol on other platform and related technical support, email to info@neo-biotech.com

7. Troubleshooting

Problem	Possible Cause	Comments/suggestions
Low or inconsistent yield	Incorrect handling of sample before cfDNA/RNA purification	For preparation, handling and storage of EDTA plasma, refer to ISO 20186-3 in order to preserve cfDNA/RNA and prevent excessive genomic DNA release. For serum and CSF handling and storage it is recommended to follow the same guidelines adopted for plasma samples.
	The sample contains low levels of cfDNA/RNA	Increase the starting sample volume as described in section 5.
	An insufficient amount of STAR BEADS Magnetic Beads was added	Vortex the tube containing the magnetic beads thoroughly immediately before use.
	Insufficient mixing of the samples with the magnetic beads during the binding step	Be sure to vortex vigorously using a vortex set at high speed for all the 10 minutes of the binding step.
	The STAR BEADS Magnetic Beads are not optimally dried	Drying times may depend on the amount of beads used and the environment. Lower volumes of beads require less time to dry. The humidity of the environment may affect the optimal bead drying time.
	Proteinase K activity reduced or lost	Do not use STAR BEADS Proteinase K after the expiration date shown on the label.
Magnetic bead carryover	Time for magnetic separation too short	Increase separation time to allow the beads to be completely attracted to the magnetic pins before aspirating any liquid from the well.
High content of gDNA into eluates	Blood sample was degraded before processing for plasma	Refers to ISO 20186-3
	Incorrect quantities of reagents used for cfDNA isolation	Make sure volumes of reagents are correctly dispensed

8. Warning and Precautions

- When working with chemicals, always wear protective equipment accessories (goggles, work clothes, hats, shoes, gloves, etc.). For more information, please refer to the appropriate safety data sheet (SDS) available online at www.neo-biotech.com
- Biological samples to be tested should be considered as potentially infectious substances and processed strictly according to laboratory biosafety requirements.
- Components from different batches cannot be used interchangeably. Do not collect reagents from other bottles of the same lot. After use, immediately close all bottles to avoid leakage, changes in buffer

concentrations or buffer contamination. After the first opening, keep all the bottles in an upright position.

- Do not use a kit after the expiration date.
- Avoid any nuclease contamination. Always wear gloves and change them often, especially after contact with skin, hair or other potentially nuclease-contaminated surfaces. Use nuclease-free solutions and nuclease- free certified, disposable plastic ware and filter tips. Maintain a separate area for nucleic acids work. Carefully clean all surfaces.

- Do not add bleach or acidic solutions directly to STAR BEADS Lysis Buffer 2, STAR BEADS Binding Buffer 2, STAR BEADS Magnetic Beads, STAR BEADS Washing Buffer 1G. They contain guanidine salts, which can form highly reactive compounds when combined with bleach. If the liquid containing these buffers is spilled, clean it with suitable laboratory detergent and water.
- Neo Biotech has not tested the liquid waste generated by the procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulations.
- In case of spillage or damage to the bottles, dispose of the components as chemical waste according to local safety regulations. Should a user detect the Product's malfunction concerning the stated specifications, download the claim form info@neo-biotech.com

STAR BEADS cfDNA/RNA Extraction Kit REF NB-78-00019-1x10

Nome: STAR BEADS Lysis Buffer 2	
Warning	
Contains:	Guanidine hydrochloride
H302+H332	Harmful if swallowed or if inhaled.
H319	Causes serious eye irritation.
P261	Avoid breathing dust / fume / gas / mist / vapours / spray.
P280	Wear protective gloves / eye protection / face protection.
P312	Call a POISON CENTRE / doctor if you feel unwell.
P264	Wash hands thoroughly after handling.

Nome: STAR BEADS Binding Buffer 2	
Danger	
Contains:	Guanidine thiocyanate
H225	Highly flammable liquid and vapour.
H302+H332	Harmful if swallowed or if inhaled.
H314	Causes severe skin burns and eye damage.
H412	Harmful to aquatic life with long lasting effects.
EUH032	Contact with acids liberates very toxic gas.
EUH071	Corrosive to the respiratory tract.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P260	Do not breathe dust / fume / gas / mist / vapours / spray.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P303+P361+P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P280	Wear protective gloves/ protective clothing / eye protection / face protection.
P310	Immediately call a POISON CENTER / doctor.

Nome: STAR BEADS Magnetic Beads	
Danger	
Contains:	Guanidine Thiocyanate
H314	Causes severe skin burns and eye damage.
EUH032	Contact with acids liberates very toxic gas.
EUH071	Corrosive to the respiratory tract.
P260	Do not breathe dust / fume / gas / mist / vapours / spray.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P303+P361+P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P280	Wear protective gloves/ protective clothing / eye protection / face protection.
P310	Immediately call a POISON CENTER / doctor.
P304+P340	IF INHALED: Remove person to fresh air and keep comfortable for breathing

Nome: STAR BEADS Proteinase K	
Danger	
Contains:	Proteinase K
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
P261	Avoid breathing dust / fume / gas / mist / vapours / spray.
P342+P311	If experiencing respiratory symptoms: call a POISON CENTER / doctor.
P304+P340	IF INHALED: remove person to fresh air and keep comfortable for breathing.

Nome: STAR BEADS Washing Buffer 1G	
Danger	
Contains:	Guanidine hydrochloride
H226	Flammable liquid and vapour.
H302+H332	Harmful if swallowed or if inhaled.
H319	Causes serious eye irritation.
H315	Causes skin irritation.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280	Wear protective gloves/ protective clothing / eye protection / face protection.
P370+P378	In case of fire: use carbon dioxide, foam, chemical powder to extinguish.
P261	Avoid breathing dust / fume / gas / mist / vapours / spray.
P312	Call a POISON CENTRE / doctor if you feel unwell.
P264	Wash hands thoroughly after handling.

Nome: STAR BEADS Washing Buffer 2G	
Warning	
H226	Flammable liquid and vapour.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280	Wear protective gloves/ protective clothing / eye protection / face protection.
P370+P378	In case of fire: use carbon dioxide, foam, chemical powder to extinguish.

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Nome: STAR BEADS Lysis Buffer 2	
Warning	
Contains:	Guanidine hydrochloride
H302+H332	Harmful if swallowed or if inhaled.
H319	Causes serious eye irritation.
P261	Avoid breathing dust / fume / gas / mist / vapours / spray.
P280	Wear protective gloves / eye protection / face protection.
P312	Call a POISON CENTRE / doctor if you feel unwell.
P264	Wash hands thoroughly after handling.

Nome: STAR BEADS Binding Buffer 2	
Danger	
Contains:	Guanidine thiocyanate
H225	Highly flammable liquid and vapour.
H302+H332	Harmful if swallowed or if inhaled.
H314	Causes severe skin burns and eye damage.
H412	Harmful to aquatic life with long lasting effects.
EUH032	Contact with acids liberates very toxic gas.
EUH071	Corrosive to the respiratory tract.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P260	Do not breathe dust / fume / gas / mist / vapours / spray.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P303+P361+P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P280	Wear protective gloves/ protective clothing / eye protection / face protection.
P310	Immediately call a POISON CENTER / doctor.

Nome: STAR BEADS Magnetic Beads	
Danger	
Contains:	Guanidine Thiocyanate
H314	Causes severe skin burns and eye damage.
EUH032	Contact with acids liberates very toxic gas.
EUH071	Corrosive to the respiratory tract.
P260	Do not breathe dust / fume / gas / mist / vapours / spray.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P303+P361+P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P280	Wear protective gloves/ protective clothing / eye protection / face protection.
P310	Immediately call a POISON CENTER / doctor.
P304+P340	IF INHALED: remove person to fresh air and keep comfortable for breathing.

Nome: STAR BEADS Proteinase K	
Danger	
Contains:	Proteinase K
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
P261	Avoid breathing dust / fume / gas / mist / vapours / spray.
P342+P311	If experiencing respiratory symptoms: call a POISON CENTER / doctor.
P304+P340	IF INHALED: remove person to fresh air and keep comfortable for breathing.

Nome: STAR BEADS Washing Buffer 1G	
Danger	
Contains:	Guanidine hydrochloride
H226	Flammable liquid and vapour.
H302+H332	Harmful if swallowed or if inhaled.
H319	Causes serious eye irritation.
H315	Causes skin irritation.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280	Wear protective gloves/ protective clothing / eye protection / face protection.
P370+P378	In case of fire: use carbon dioxide, foam, chemical powder to extinguish.
P261	Avoid breathing dust / fume / gas / mist / vapours / spray.
P312	Call a POISON CENTRE / doctor if you feel unwell.
P264	Wash hands thoroughly after handling.

Nome: STAR BEADS Washing Buffer 2G	
Warning	
H226	Flammable liquid and vapour.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280	Wear protective gloves/ protective clothing / eye protection / face protection.
P370+P378	In case of fire: use carbon dioxide, foam, chemical powder to extinguish.

Contact and technical support information: info@neo-biotech.com

9. Ordering information

PRODUCT	ORDER - N	COMPATIBLE EXTRACTORS	UNIT SIZE
STAR BEADS cfDNA/RNA Extraction Kit	NB-78-00019-1x10 NB-78-00019-1x96	Auto-Pure 24, MOLGEN PurePrep 24, liquid handling systems	10 preps 96 preps

For further information

Visit: www.neo-biotech.com