NeoGeneStar
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www.NeoGeneStar.com

NeoGeneStar™ Cell Free DNA Purification Kit for 2.5ml Samples

For purification of cell-free DNA from 2.5 ml of plasma, serum, CSF or urine

For Research Use Only.

Not for human or animal therapeutic or diagnostic use.

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Binding Characteristics and Sample Volume

The superparamagnetic particles bind DNA molecules from ~30 bases to > 10,000 bases. The 2.5ml size NeoGeneStar™ circulating cell-free DNA Kit has been optimized for sample volumes of up to 2.5ml.

Catalog No	Sample Volume and Quantity	Pretreatment Buffer (20x)	NGS Protease (25X)	RNA Carrier	LYS[1] Tubes	NGS™ Beads	Wash Buffer[2]	Elution Buffer
NeoGeneStar™2.5ml-25-WPR	2.5ml x 25 preps	3.125ml	2.5ml	125µl	25	0.75 ml	40ml	2..5ml
NeoGeneStar™2.5ml-50-WPR	2.5ml x 50 preps	6.25ml	5ml	250µl	50	1.5ml	80ml	5.0ml
NeoGeneStar™2.5ml-100-WPR	2.5ml x 100 preps	12.5ml	10ml	500µl	100	3.0ml	160ml	10.0ml

[1]LYS tubes contain chaotropic salts, which are irritants. Please wear gloves and handle with appropriate laboratory safety measures.
[2]Absolute ethanol must be added at 1:1 ratio prior to use for the Wash Buffer.

Procedure of the NeoGeneStar™ Circulating cfDNA Kit

For 2.5ml sample, add 0.125ml Pretreatment Buffer, 0.1ml NGS Protease, and 5µl RNA carrier to 2.5ml sample in a 15 ml centrifuge tube, incubate 30 minutes at 55-60ºC.

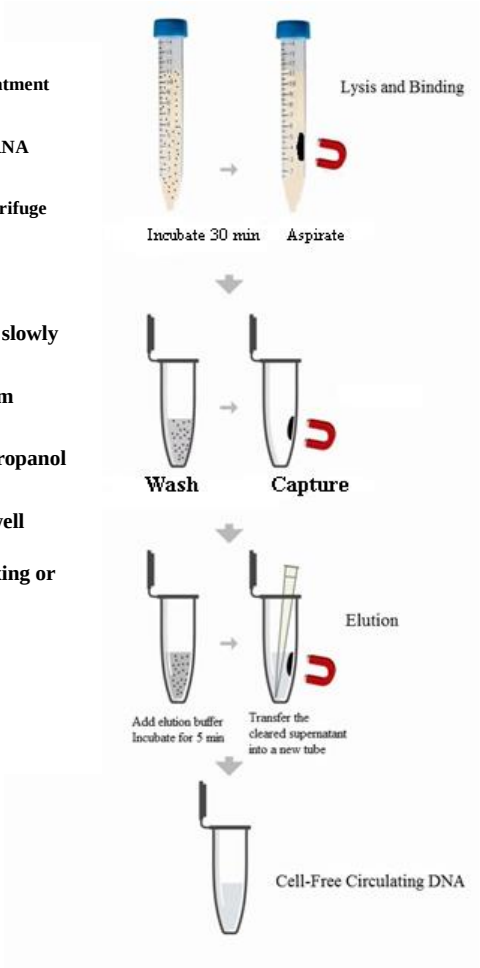
Add the entire pretreated plasma slowly into the LYS tube, dissolve at room temperature, then add 0.5ml isopropanol and 30µl NGS™ Beads and mix well

Incubate for 30 minutes by vortexing or inverting

Wash 2 times with Wash Buffer

Wash 2 times with 80% Ethanol

Elute



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Note Regarding Wash Buffer

Wash Buffer must be diluted with an equal amount of absolute ethanol and stored at room temperature prior to use. In the event that the Wash Buffer is chilled, a precipitate may form; it would then be necessary to warm the Wash Buffer to fully dissolve the components prior to use.

Materials Needed That Are Not Supplied:

- Low DNA binding microcentrifuge tubes
- Normal Saline (0.9% w/v NaCl) solution (for volume adjustment of samples less than 2.5ml)
- Magnetic separation devices (for 15ml and microcentrifuge tubes)
- Absolute ethanol and isopropanol
- Tube shaker / vortexer for 15ml and microcentrifuge tubes.

Purification Protocol:

1. For 2.5ml plasma, add 0.125ml Pretreatment buffer, 0.1ml of NGS Protease, and 5µl RNA carrier in a 15ml centrifuge tube, mix well and incubate at 55-60^{o}C for 30 minutes.

   Please note: If the sample volume is less than the designed kit processing volume, add the appropriate volume of 0.9% sodium chloride solution (normal saline) to bring the volume to the specified sample volume. (ie. if the sample volume is 2ml, add 0.5ml of 0.9% sodium chloride solution to result in a 2.5ml volume for processing sample).
2. Add the entire pretreated sample to the 2.5ml LYS tube and mix thoroughly at room temperature, ensure that the reagents are fully dissolved, then add 0.5ml isopropanol, mix.
3. **Resuspend the NGS™ Beads by vortexing for 1 minute**, then add 30µl of NGS™ Beads, vortex or invert for 30 minutes on a vortexer or 360^{o} rotator. Proper shaking will result in visible foam above the liquid layer.
4. Centrifuge briefly to reduce the foam, then place the 15ml tube on a magnetic stand for at least 2 minutes, the solution will clear. With the sample tube on the magnetic stand, carefully aspirate the cleared supernatant without aspirating the NGS™ Beads. Vacuum aspiration is convenient but not necessary.
5. Remove the sample tube from the magnetic stand, add 1ml of Wash Buffer (diluted 1:1 with absolute ethanol) into the tube and carefully rinse the NGS™ Beads and transfer to a 2.0ml low DNA binding microcentrifuge tube, add 0.4ml diluted Wash Buffer to rinse the 15ml tube again and transfer to the microcentrifuge tube.
6. Place the microcentrifuge tube on a magnetic stand for at least 1 minute or until the solution clears, turn the tube over a few times while on the magnetic stand to remove the NGS™ Beads trapped on the lid. With the sample tube on the magnetic stand, carefully

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aspirate the cleared supernatant without aspirating the NGS™ Beads.

7. Remove the microcentrifuge tube from the magnetic stand and add 1ml of Wash Buffer (diluted 1:1 with absolute ethanol) into the tube and resuspend the NGS™ Beads by vortexing for 30 seconds.
8. Place the sample tube onto the magnetic stand for at least 1 minute or until the solution clears, turn the tube over a few times while on the magnetic stand to remove the NGS™ Beads trapped on the lid. With the sample tube on the magnetic stand, carefully aspirate the cleared supernatant without aspirating the NGS™ Beads.
9. Remove the microcentrifuge tube from the magnetic stand and add 1ml of 80% ethanol to the tube. Completely resuspend the NGS™ Beads by vortexing for 30 seconds.
10. Place the microcentrifuge tube on the magnetic stand for at least 1 minute or until the solution clears, turn the tube over a few times while on the magnetic stand to remove the NGS™ Beads trapped on the lid. With the sample tube on the magnetic stand, carefully aspirate the cleared supernatant without aspirating the NGS™ Beads.
11. Wash the NGS™ Beads with 1ml of 80% ethanol again by repeating steps 9 and 10.
12. Pulse down and aspirate again to remove as much of the liquid as possible. Keeping the tube on the magnet, air-dry the NGS™ Beads at room temperature for 10 minutes.
13. Add 30µl-100µl Elution Buffer or elution buffer of your choice and resuspend the NGS™ Beads by pipetting up and down, then incubate 10 minutes at room temperature.
14. Place sample tubes on a magnetic stand, the solution will clear in about 1 minute. Transfer the cleared supernatant into a low DNA binding microcentrifuge tube. This is the purified cfDNA. Freeze the eluted cfDNA until you are ready for your downstream analysis.

Note: For certain applications, such as digital (microdroplet) PCR, it may be advantageous to briefly microcentrifuge to remove any NGS™ Beads that maybe present in the eluate.

The information in this guide is subject to change without notice.

Patent pending.

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