NeoGeneStar
100 Randolph Road, Suite 2B
Somerset, NJ 08873
Tel: (732) 421-4567
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www.NeoGeneStar.com

NeoGeneStar™ Cell Free DNA Purification Kit for 8ml Samples

For purification of cell-free DNA from 8ml 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 8ml size NeoGeneStar™ circulating cell-free DNA Kit has been optimized for sample volumes of up to 8ml.

Catalog No	Sample Volume and Quantity	Pretreatment Buffer (20x)	NGS Protease (20X)	RNA Carrier	LYS[1] Tubes	NGS™ Beads	Wash Buffer[2]	Elution Buffer
NeoGeneStar™8ml-25-WPR	8ml x 25 preps	10ml	8ml	125µl	25	1.25 ml	40ml	2..5ml
NeoGeneStar™8ml-50-WPR	8ml x 50 preps	20ml	16ml	250µl	50	2.5ml	80ml	5.0ml
NeoGeneStar™8ml-100-WPR	8ml x 100 preps	40ml	32ml	500µl	100	5.0ml	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 8ml sample, add 0.4ml Pretreatment Buffer, 0.32ml NGS Protease, and 5µl RNA carrier to 8ml sample in a 15ml centrifuge tube, incubate 30 minutes at 55-60ºC.

Add the entire pretreated plasma slowly into the LYS tube, dissolve at room temperature, then add 1.6ml isopropanol and 50µl NGS™ Beads and mix well

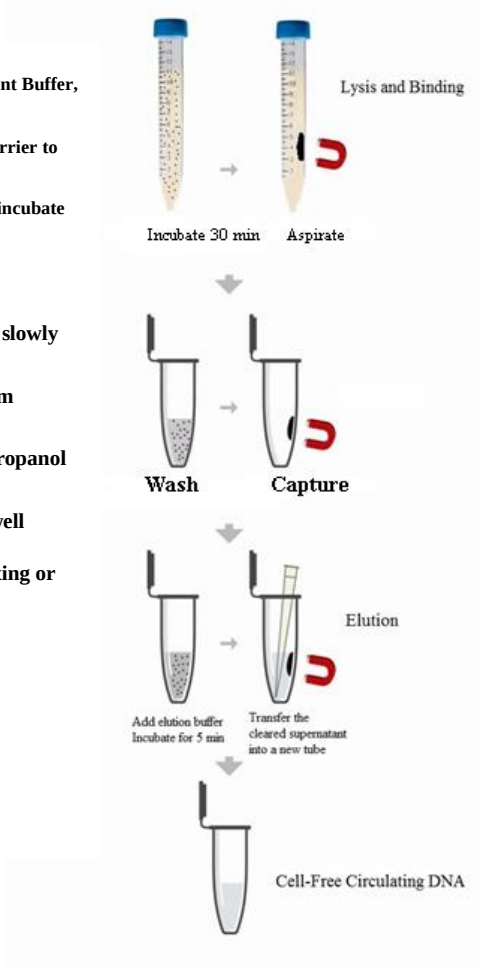
Incubate for 30 minutes by vortexing or inverting

Wash 2 times with Wash Buffer

Wash 2 times with 80% Ethanol

Air dry 5-10 minute

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 8ml)
- Magnetic separation devices (for 50ml and microcentrifuge tubes)
- Absolute ethanol and isopropanol
- Tube shaker / vortexer for 50ml and microcentrifuge tubes.

Purification Protocol:

1. For 8ml plasma, add 0.4ml Pretreatment buffer, 0.32ml 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 7.5ml, add 0.5ml of 0.9% sodium chloride solution to result in a 8ml volume for processing sample).
2. Add the entire pretreated sample to the 8ml LYS tube and mix thoroughly at room temperature, ensure that the reagents are fully dissolved, then add 1.6ml isopropanol, mix.
3. **Resuspend the NGS™ Beads by vortexing for 1 minute**, then add 50µl 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 50ml 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 50ml 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 50µl-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.