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NextPrep-Mag™ cfDNA Isolation Kit

(For 3 mL - 5 mL Plasma Samples)

Catalog #NOVA-3825-03 (Kit contains 50 Isolations)

 V18.12

This product is for research use only.
Not for use in diagnostic procedures.

NEXTprep-Mag™ cfDNA Isolation Kit - NOVA-3825-03

Product Overview

The NextPrep-Mag™ cfDNA Isolation Kit For 3 mL - 5 mL Plasma Samples is designed for extracting cell-free DNA (cfDNA) using a magnetic bead format. The procedure, which can be completed in approximately 25 minutes, consists of an initial protease digestion/DNA binding step, wash steps, and elution of the cfDNA from the magnetic beads. Suitability of the DNA for use in NGS library construction has been verified using the Bioo Scientific NEXTflex® Cell Free DNA-Seq Kit (cat # 5150).

Contents, Storage and Shelf Life

The NextPrep-Mag™ cfDNA Isolation Kit For 3 mL - 5 mL Plasma Samples contains sufficient reagents for extraction of DNA from 50 samples of 3 mL - 5 mL plasma. The kit components are stable for at least 12 months from time of receipt, when properly stored.

Kit Contents	Amount	Storage
cfDNA Proteinase K	7 mL in 50% glycerol buffer	-20°C
cfDNA Binding Solution	350 mL	Room Temp
cfDNA Magnetic Beads	5 mL	Room Temp
cfDNA Wash 1	170 mL	Room Temp
cfDNA Wash 2	45 mL (user adds 135 mL ethanol)	Room Temp
cfDNA Elution Solution	6 mL	Room Temp

Revision History

Version	Date	Description
V15.03	March 2015	Initial Product Launch.
V15.05	May 2015	The volume of Control PCR Primers has been reduced from .2 mL to 24 µL.
V15.07	July 2015	Wash and elution procedure has been optimized.
V16.01	January 2016	The protocol has been optimized for plasma samples 3 mL - 5 mL in volume. Users with smaller samples should use Cat. # 3825-01, designed for samples that are < 1 mL - 3 mL in volume. The Control PCR Primers are no longer included.
V18.12	December 2018	Comment about pre-mixing Proteinase K with Binding Solution removed and comment about optional secondary elution step and elution buffer added.

Required Materials Not Provided

Reagents:

- Ethanol, 100%

Equipment and Supplies:

- Vortex mixer
- Equipment for continuous agitation of prep (for example rotary mixer, platform shaker, or rocking mixer) or heat block at 55°C
- Magnetic stands to hold 2 mL microcentrifuge tubes and 15 mL tubes
- Microcentrifuge or low-speed "picofuge"
- Nuclease-free 2 mL (1.5 mL and 0.5 mL optional) microcentrifuge tubes and 15 mL tubes
- Pipettors (P-1000, P-200, P-20) and tips.
- 5 mL disposable pipettes

Warnings and Precautions

Bioo Scientific recommends that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local distributor, or Bioo Scientific at BiooNGS@perkinelmer.com.

- When handling Binding Solution and Proteinase K, wear protective gloves, protective clothing, eye protection and face protection. Avoid contact with skin.
- The Binding Solution and Wash 1 contain guanidinium salts and should not be mixed with bleach.
- Human blood products should be handled and disposed of using universal precautions for working with biohazardous material.

Reagent Preparation

Before using the kit the first time:

1. Prepare working Wash 2 by adding 135 mL of 100% ethanol and mixing thoroughly. Place check mark in box on label to indicate ethanol has been added. Store at room temperature.
2. optional: The Elution Solution in this kit is 5 mM Tris pH 8/0.1 mM EDTA / 50 mM NaCl. The lab may substitute this Elution Solution using their own 10 mM Tris pH 8/ 0.1 mM EDTA solution depending on their application needs.

cfDNA Isolation

Materials

Bioo Scientific Supplied
NextPrep-Mag™ cfDNA Isolation Kit

User Supplied
Magnetic Stand for 15 mL tubes
Magnetic Stand for 2 mL tubes
Vortex Mixer
Heat Block at 55°C

The following table lists the volumes of the components to be used, depending on the starting plasma sample volume.

Component	Volume to use with respect to plasma volume	Example for 3 mL Plasma Sample	Example for 4 mL Plasma Sample	Example for 5 mL Plasma Sample
Binding Solution	1.25X	3.75 mL	5 mL	6.25 mL
Proteinase K	0.024X	72 µL	96 µL	120 µL
Magnetic Beads	0.016X	48 µL	64 µL	80 µL
Elution Solution*	0.012X	36 µL	48 µL	60 µL

* Elution Solution volumes shown are the minimum; higher volumes of Elution Solution may be used.

1. **Mix plasma sample with the indicated volume of Binding Solution** in a 15 mL tube. Vortex briefly, then add **the indicated volume of Proteinase K.** Vortex briefly, then add **the indicated volume of well-mixed Magnetic Beads**. Mix thoroughly by vigorous pulse-vortexing for ~10 seconds. *Note: The Binding Solution and Magnetic Beads can be assembled as a Master Mix and added as a single component. The Proteinase K must be added separately.*
2. Incubate for **15 minutes with continuous agitation (for example on rotary mixer or platform shaker) or at 55°C in a heat block**.
3. Vortex or invert tube briefly to remix any beads that have settled, then place tube on magnetic stand for ~30 seconds, or as long as needed to completely attract beads. Thoroughly remove and discard the supernatant.
4. Resuspend beads in **1.5 mL of Wash 1** by vortexing, then transfer slurry to a 2 mL tube. Avoid transferring foam from bottom of tube.
5. Place tube on magnetic stand for ~5 seconds, or as needed. Remove and discard supernatant.

6. Resuspend beads **for a second time in 1.5 mL of Wash 1** by vortexing. Place tube on magnetic stand for ~5 seconds, or as needed. Remove and discard the supernatant.

7. Resuspend beads in **1.5 mL of Wash 2** by vortexing. Place tube on magnetic stand for ~5 seconds, or as needed. Remove and discard the supernatant.

8. Resuspend beads **for a second time in 1.5 mL of Wash 2** by vortexing. Place tube on magnetic stand for ~5 seconds, or as needed. Remove and discard the supernatant.

9. Pop-spin tube to collect residual fluid at bottom of tube, then return tube to magnet for ~5 seconds, or as needed. Remove and discard the supernatant.

10. Elute cfDNA by adding **at least the minimum volume of Elution Solution indicated in the table on the previous page**, then resuspend beads by vortexing. Tap tube or pop-spin if needed to collect slurry at bottom of tube. *Note: Volume of Elution Solution may be increased if desired. When choosing an elution volume, please note that there is a trade-off between maximizing recovery and maximizing concentration. Eluting in the minimum volume may result in lower recovery, but will maximize the concentration of the sample.*

11. Incubate for **5 minutes with continuous agitation or at 55°C in a heat block**.

optional: To increase total yield, the lab may optimize the elution steps and include a second elution from the same bead. Contact bioo.ngs@perkinelmer.com for additional information.

12. Tap tube or pop-spin if needed to collect all liquid and bead slurry at bottom of tube. Place tube on magnetic stand for ~5 seconds, or as needed.

13. Transfer the cfDNA eluate to fresh tube. Store at -20°C.

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WE WANT TO HEAR FROM YOU!

Your feedback is important to us. Tell us what you think of our kits by scanning the QR code or visiting our website at www.biooscientific.com/NGSfeedback. We can't wait to hear from you!

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THE NGS EXPERTS™

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