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FOR REFERENCE PURPOSES

This manual is for Reference Purposes Only. DO NOT use this protocol to run your assays. Periodically, optimizations and revisions are made to the kit and protocol, so it is important to always use the protocol included with the kit.

NextPrep-Mag™ Urine cfDNA Isolation Kit

(For < 1 mL - 4 mL Urine Samples)

Catalog #3826-01 (12 - 50 Isolations)

 V16.04

This product is for research use only.

NextPrep-Mag™ Urine cfDNA Isolation Kit - 3826-01

Product Overview

The NextPrep-Mag Urine cfDNA Isolation Kit is designed for extracting cell-free DNA (cfDNA) from urine 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 NEXTflex™ Cell Free DNA-Seq Kit (Cat # 5150).

Contents, Storage and Shelf Life

The NextPrep-Mag Urine cfDNA Isolation Kit For < 1 mL - 4 mL Urine Samples is fully scalable for use with any sample volume of urine between ~ 300 µL and 4 mL. The number of isolations possible is inversely proportional to the volume of urine sample used. For example, this kit contains sufficient reagents for extraction of DNA from 50 samples of up to 1 mL urine, or 12 samples of 4 mL urine. The kit components are stable for at least 12 months from time of receipt, when properly stored.

Kit Contents	Amount	Stroage
cfDNA Proteinase K	400 µL in 50% glycerol buffer	-20°C
Urine cfDNA Binding Solution	50 mL	RT
cfDNA Magnetic Beads	300 µL	RT
cfDNA Wash 1	150 mL	RT
cfDNA Wash 2	42 mL (user adds 126 mL ethanol)	RT
cfDNA Elution Solution	1.8 mL	RT
Control PCR primers	100 µL (50 uM For + 50 uM Rev)	-20°C

Note: Do not freeze the Magnetic Beads.

Required Materials Not Provided

Reagents:

- Ethanol, 100%

Equipment and Supplies:

- Vortex mixer
- Heat block at 55°C
- Magnetic stands to hold 2 mL microcentrifuge tubes and 15 mL or 50 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.
- Disposable pipettes (variable sizes depending on sample volume processed)

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@biooscientific.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 bodily fluids should be handled and disposed of using universal precautions for working with potentially biohazardous material.

Reagent Preparation

Before using the kit the first time:

1. Prepare working Wash 2 by adding 126 mL of 100% ethanol and mixing thoroughly. Place check mark in box on label to indicate ethanol has been added. Store at room temperature.

cfDNA Isolation

Materials

Bioo Scientific Supplied
NextPrep-Mag Urine cfDNA Isolation Kit

User Supplied
Magnetic Stand for 15 mL or 50 mL tubes
Magnetic Stand for 2 mL tubes
2 mL microfuge tubes; optional, smaller microfuge tubes for elution of cfDNA
15 mL or 50 mL tubes (for preps > 0.9 mL urine)
Vortex Mixer
Heat Block at 55°C

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

Component	Volume to use with respect to urine volume	Example for 1mL urine	Example for 2 mL urine	Example for 3 mL urine	Example for 4 mL urine
Urine cfDNA Binding Solution	1.0 X	1 mL	2 mL	3 mL	4 mL
Proteinase K	0.008 X	8 µL	16 µL	24 µL	32 µL
Magnetic Beads	0.006 X	6 µL	12 µL	18 µL	24 µL
Elution Solution*	At least 0.003 X	3 µL	6 µL	9 µL	12 µL

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

Recommended: To minimize contamination of the urine sample with cells from the donor's urinary tract, collect clean-catch urine (do not collect first ~ 10 mL). Transfer the urine to a 15 mL or 50 mL tube and centrifuge for 15 minutes at 3,000 rcf at room temp, then remove supernatant to fresh tube, leaving ~ 0.5 mL residual urine behind.

1. Mix urine sample with the **indicated volume of Urine cfDNA Binding Solution** in tube of appropriate size. 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 ~15 seconds. *Note: The Binding Solution, Proteinase K, and Magnetic Beads can be assembled as a Master Mix and added as a single component.*

2. Incubate for **15 minutes at 55°C**.

3. Place tube on magnetic stand for ~3 minutes, or as long as needed to completely attract beads. Thoroughly remove and discard the supernatant. *Note: Preps using higher volumes of urine may require a few minutes to completely attract the beads, whereas lower-volume preps may require less time.*

4. Resuspend beads in **1.5 mL of Wash 1** by vortexing, then transfer slurry to a 2 mL tube.

5. Place tube on magnetic stand for ~10 seconds, or as needed to completely attract beads. 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 ~10 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 ~10 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 ~10 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 residual fluid.

10. Elute cfDNA by adding **at least the minimum volume of Elution Solution indicated in the table**, 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.

11. Incubate for **5 minutes at 55°C**.

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 ~10 seconds, or as needed.

13. Transfer the cfDNA eluate to fresh tube. To maximize recovery of eluate, for preps eluted in very low volumes, re-spin tube ~3 seconds, replace on magnet, remove residual fluid and add to cfDNA eluate. Store cfDNA at -20°C.

Verification of cfDNA Recovery Using Control Primers

The control primers are designed to produce a 140 bp amplicon from an X-linked single-copy human gene.

Typical 20 µL reaction:

Component	Volume to Add
Input cfDNA	4 µL
5X PCR MasterMix	4 µL
Control Primers from kit	2 µL
Water (distilled deionized)	10 µL

PCR Amplification

1. Suggested amplification protocol:

4 min	95°C
20 sec	95°C
20 sec	55°C
40 sec	72°C
5 min	72°C

} *Cycle ~30X (required number of PCR cycles depends on volume of urine processed, volume of elution solution used, and sample-specific differences in concentration of urine cfDNA)

Note 1: 5X PCR MasterMix contains thermostable polymerase, 4dNTPs, and buffer. As an alternative to 5X PCR MasterMix, these components may be added separately. Optionally, the MasterMix may also include gel-loading components (tracking dye and density component) to allow the PCR to be loaded directly on a gel. Loading directly helps to minimize chance of contamination in negative controls in subsequent reactions.

Note 2: It is recommended to include a negative control PCR containing Elution Solution and/or water instead of input cfDNA. It is suggested to also include at least one positive control of cfDNA known to support PCR. A dilute solution of human genomic DNA may also be used as a positive control.

Note 3: The above conditions are an example and may be altered according to experience based on results with positive and negative control reactions. Using higher numbers of cycles increases the risk of contamination in the negative controls.

PCR products may be assessed by running the entire 20 µL PCR on a 2% agarose gel in Tris/Borate/EDTA (TBE) buffer. The amplicon may be visualized by ethidium bromide (EtBr) staining. For convenience, the EtBr may be added to the TBE running buffer and the gel.

RELATED PRODUCTS

Illumina Compatible Cell Free DNA NGS Kits & Adapters

Catalog #	Product
5150-01	NEXTflex™ Cell Free DNA-Seq Kit (8 reactions)
5150-02	NEXTflex™ Cell Free DNA-Seq Kit (48 reactions)
514101	NEXTflex™ DNA Barcodes - 6
514102	NEXTflex™ DNA Barcodes - 12
514103	NEXTflex™ DNA Barcodes - 24
514104	NEXTflex™ DNA Barcodes - 48
514105	NEXTflex-96™ DNA Barcodes - 96
514160	NEXTflex™ Dual-Indexed DNA Barcodes (1-96)
514161	NEXTflex™ Dual-Indexed DNA Barcodes (97-192)
3825-01	NextPrep-Mag™ cfDNA Isolation Kit (< 1 mL - 3 mL)
3825-03	NextPrep-Mag™ cfDNA Isolation Kit (3 mL – 5 mL)

Ion PGM & Ion Proton Compatible Cell Free DNA NGS Kits & Adapters

Catalog #	Product
5150-01	NEXTflex™ Cell Free DNA-Seq Kit for Ion PGM & Ion Proton (8 reactions)
5150-02	NEXTflex™ Cell Free DNA-Seq Kit for Ion PGM & Ion Proton (48 reactions)
514101	NEXTflex™ DNA Barcodes for Ion PGM & Ion Proton- 8
514102	NEXTflex™ DNA Barcodes for Ion PGM & Ion Proton- 16
514103	NEXTflex™ DNA Barcodes for Ion PGM & Ion Proton- 32
514104	NEXTflex™ DNA Barcodes for Ion PGM & Ion Proton- 64

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