

## Instructions for Use

# Revolution cfDNA Max 50 Kit™ Revolution cfDNA Max 50 Reagent Kit™

## Version A

For in vitro diagnostic use only



405000, 404000



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POWERING LIQUID BIOPSY

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## **Intended Use**

The Revolution cfDNA Max 50 Kit and Revolution cfDNA Max 50 Reagent Kit are intended to extract cell-free DNA (cfDNA) from human plasma and urine.

The kits are intended to be used by physicians, researchers, and technicians who have received training in molecular biology laboratory techniques.

These kits are intended for in vitro diagnostic use only, and only when the Revolution Plus instrument is operating in “Kit Mode” with the latest version of the Revolution Plus firmware installed.

## **Summary and Explanation**

The Revolution cfDNA kits employ well-characterized technology to extract cfDNA from large volume plasma and urine samples. The kit procedures are designed so users can process multiple samples simultaneously.

The procedures are suitable for nucleic acid isolation from human cell-free plasma or urine. Samples can be either fresh or frozen, although it is recommended that samples that have been previously frozen and thawed are not frozen again. The procedures are designed for minimal user handling. Laboratory gloves and standard infection-control procedures are recommended while handling biological materials

The isolated cfDNA is ready for use in downstream applications, including PCR, real-time PCR (RT-PCR), and Next-Generation Sequencing (NGS). Alternatively, the purified cfDNA can be stored at -80°C to -20°C for later use.

# **Principles of the Procedure**

Each Revolution cfDNA kit procedure includes the following steps:

- Digest proteins in the plasma or urine sample and protect cfDNA from degradation
- Bind the cfDNA in the plasma or urine sample to magnetic beads
- Capture the beads by magnetic separation
- Wash the beads
- Elute the cfDNA from the magnetic beads

## **Protease Treatment**

Enzymes and other proteins are digested in the Revolution nRicher Cartridge's Sample Tube (see Figure 1 on page 7 for a diagram of the Revolution nRicher Cartridge and its parts).

## **Addition of Antifoaming Agent**

An antifoaming agent is added to each sample to minimize foam formation during the Bead Binding and Bead Capture steps.

## **Bead Binding**

Revolution cfDNA Magnetic Beads and Revolution cfDNA Binding Buffer are combined with the sample in the Revolution nRicher Cartridge's Sample Tube, and then placed into the Revolution Plus Processor, and incubated to allow the magnetic beads to capture the cfDNA in the sample.

## **Bead Capture**

The Revolution Mag Capsule is attached to the Revolution nRicher Cartridge's Capture Tube, which is again placed into the Revolution Plus Processor and incubated to capture the beads in the Capture Tube portion of the nRicher Cartridge.

## **Bead Washing**

The Capture Tube is removed from the nRicher Cartridge and washed twice with Revolution cfDNA Wash Solution and then rinsed first with 100% ethanol, followed by 80% ethanol using the Revolution Mag Rack to capture the magnetic beads after each wash/rinse. The beads are then dried.

## **Elution**

The cfDNA is eluted from the beads with Revolution cfDNA Elution Buffer and is ready for downstream applications.

## **Materials Provided**

**IMPORTANT:** Upon receipt of the kits, remove the Protease Powder and Magnetic Beads from the kit and store them at the temperatures indicated on the component labels and package insert. All other kit components may be stored at typical indoor temperatures of 15 °C - 30 °C.

### **Revolution cfDNA Max 50 Kit, (# 405000)**

- Revolution cfDNA Max 50 Surfactant, 45 mL, 414000
- Revolution cfDNA Max 50 Lysis Buffer, 125 mL, 420000<sup>†</sup>
- Revolution cfDNA Max 50 Protease Powder, 250 mg, 422000<sup>‡</sup>
- Revolution cfDNA Max 50 Protease Buffer, 14 mL, 418000
- Revolution cfDNA Max 50 Binding Buffer, 2 x 500 mL, 410000<sup>†</sup>
- Revolution cfDNA Max 50 Antifoaming Agent, 500 µL, 410080
- Revolution cfDNA Max 50 Magnetic Beads, 2 x 4 mL, 424000<sup>‡</sup>
- Revolution cfDNA Max 50 Wash Solution, 60 mL, 412000
- Revolution cfDNA Max 50 Elution Buffer, 4 mL, 416000
- Revolution nRicher Cartridge 100 mL Sample Tubes, 4 packs of 6 Sample Tubes, 450010
- Revolution nRicher Cartridge Capture Tubes, 3 packs of 8 Capture Tubes, 450003
- Revolution Capture Tube Caps, Qty. 100, 400063
- Instructions for Use (This document. The most current version is available at [www.nrichdx.com](http://www.nrichdx.com))

### **Revolution cfDNA Max 50 Reagent Kit, (# 404000)**

- Revolution cfDNA Max 50 Surfactant, 45 mL, 414000
- Revolution cfDNA Max 50 Lysis Buffer, 125 mL, 420000<sup>†</sup>
- Revolution cfDNA Max 50 Protease Powder, 250 mg, 422000<sup>‡</sup>
- Revolution cfDNA Max 50 Protease Buffer, 14 mL, 418000
- Revolution cfDNA Max 50 Binding Buffer, 2 x 500 mL, 410000<sup>†</sup>
- Revolution cfDNA Max 50 Antifoaming Agent, 500 µL, 410080
- Revolution cfDNA Max 50 Magnetic Beads, 2 x 4 mL, 424000<sup>‡</sup>
- Revolution cfDNA Max 50 Wash Solution, 60 mL, 412000
- Revolution cfDNA Max 50 Elution Buffer, 4 mL, 416000
- Instructions for Use (This document. The most current version is available at [www.nrichdx.com](http://www.nrichdx.com))

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<sup>†</sup> Contains guanidinium thiocyanate. Do not combine with disinfectants that contain bleach. See “Warnings and Precautions” section of this document for more information.

<sup>‡</sup> Upon receipt, store the Protease Powder frozen at -25 °C to -15 °C and the Magnetic Beads at the temperature indicated on the Magnetic Beads label and Package Insert. All other kit components may be stored at ambient temperature 15 ° to 25 ° C (59 ° to 77 ° F).

## Other Materials Required (Not Provided)

Always wear personal protective equipment (PPE), such as a lab coat, protective eyewear, and disposable gloves when working with chemicals. Consult the appropriate Safety Data Sheets (SDSs for Revolution Kits are available at <https://www.nrichdx.com/material-data-safety-sheets>; other SDSs are available from the product supplier) for more information on safe handling and use.

### Revolution Plus Sample Prep System Equipment

- Drip Tray, 100291
- Revolution Cartridge Rack, 200600
- Revolution Mag Capsules, 200700
- Revolution Capture Tube Mag Rack, 200800
- Revolution Plus Processor, 2000PLUS

### Additional Materials and Reagents

- 100% ethanol
- 80% ethanol
- Centrifuge and Microcentrifuge<sup>†</sup>
- Non-magnetic microvial rack\* (**NOTE:** Capture Tube will generally fit a microvial tube rack)
- Phosphate Buffered Saline (PBS) pH 7.5
- Pipettors<sup>†</sup>, pipette tips<sup>‡</sup>, and serological pipettes
- Vortex instrument with 2 mL microvial tube adaptor\*

**\*NOTE:** The Capture Tube of the nRicher Cartridge is sized to fit most standard non-magnetic microvial racks and microvial tube adaptors on common vortex instruments.

## nRicher Cartridge Usage and Handling

The nRicher Cartridge is comprised of two parts - the Sample Tube and the Capture Tube as shown in Figure 1. To join them, push the Capture Tube's open end evenly into the open end of the Sample Tube until you hear a click sound and physical sensation as shown in Figure 2. To remove the Capture

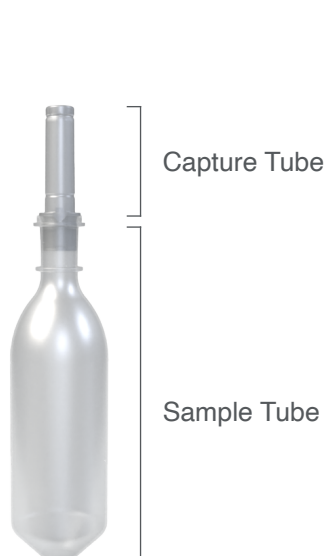
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<sup>†</sup> We strongly recommend that instruments are calibrated at regular intervals to ensure that samples are processed consistently and accurately

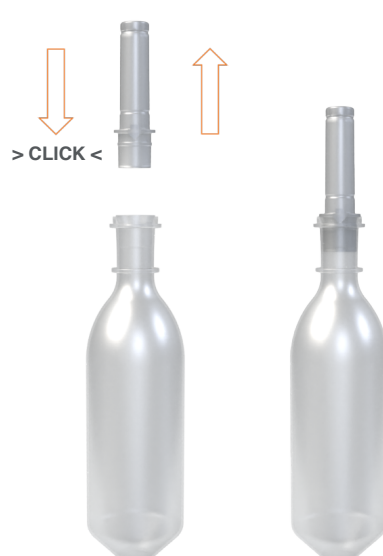
<sup>‡</sup> We strongly recommend using pipette tips with aerosol barriers to prevent cross-contamination.

Tube, pull the Capture Tube evenly away from the Sample Tube as shown in Figure 2.

Similarly, the Revolution Mag Capsule is added to the Capture Tube by placing the Mag Capsule's larger opening over the top of the Capture Tube and pushing down until the Mag Capsule is fully seated on the Capture Tube as shown in Figure 3. Note: a slight clockwise/counterclockwise twisting of the Mag Capsule until the Mag Capsule is fully seated may be required. To remove the Mag Capsule, pull the Mag Capsule evenly away from the Capture Tube as shown in Figure 3.



**Figure 1 - nRicher Cartridge**  
The nRicher Cartridge is comprised of the Sample Tube and the Capture Tube



**Figure 2 - Capture Tube**  
Joining or separating the Capture Tube and Sample Tube



**Figure 3 - Mag Capsule**  
Joining or separating the Mag Capsule and Capture Tube from the Sample Tube



**Figure 4 - Releasing the Capture Tube from the Mag Capsule**  
Place the Mag Capsule with the open end of the Capture Tube facing upward on a laboratory benchtop and press down; the Capture Tube will click as it moves upward slightly and is released from the Mag Capsule. The Capture Tube may now be removed from the Mag Capsule by gripping the Capture Tube and pulling it upward gently away from the Mag Capsule.



**Figure 5 - Magnetic Rack**  
12-position Capture Tube Magnetic Rack (Mag Rack)



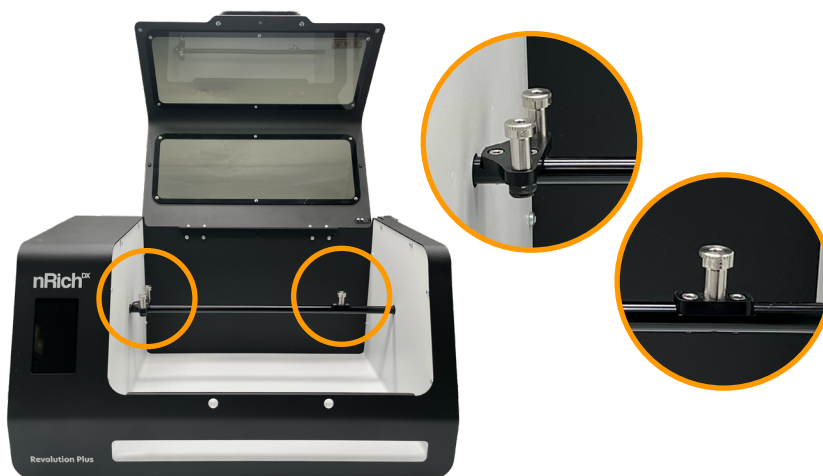
**Figure 6 - Cartridge Rack with Cartridges**  
nRicher Cartridges shown in the Cartridge Rack; the Capture Tube is removed and placed in a separate rack when accessing the Sample Tube portion of the nRicher Cartridge.



**Figure 7 - Rack with Cartridges, Mag Capsules**  
nRicher Cartridges with Mag Capsules attached and positioned in the Cartridge Rack.



**Figure 8**  
The Revolution Plus Processor. Arrow indicates placement of the Drip Tray. Do not attempt to remove the drip tray once the Cartridge Rack with Cartridges is in place, or the instrument is in operation.



**Figure 9**  
The Revolution Plus Processor - door open. Note the Cartridge Rack attachment rod and pins - two pins on the left side of the rod and one pin on the right. Pins attach the Cartridge Rack to the rod.



**Figure 10 - Placing the Cartridge Rack into, and removal from, the Revolution Plus Processor**

Two pin slots on the left-hand side of the Cartridge Rack and one pin slot on the right-hand side securely attach the rack to the processor as follows: Grip the Cartridge Rack handles and place the Cartridge Rack directly over and resting on the attachment rod so the pins are to the left and adjacent to the slots. Slide the rack to the left into the pins on the attachment rod until the pins are attached to the Cartridge Rack and the single pin is positioned where indicated by the red arrow shown above. To remove the Cartridge Rack, first ensure all motion of the Rack has stopped, grip the Cartridge Rack handles and slide the rack to the right until the pins have detached from the Cartridge Rack's slots. Lift the rack over the attachment rod and free from the Revolution Plus Processor.



## Reagent Storing and Handling

The Revolution cfDNA kits are shipped at room temperature.

**IMPORTANT:** Upon arrival, remove the components indicated below and store them at the indicated storage temperatures:

- Revolution cfDNA Protease Powder should be stored at -25 °C to -15 °C and can be used until the kit expiration date without affecting protease performance.
- Revolution cfDNA Magnetic Beads should be stored at the temperature indicated on the label and the package insert.
- All other kit components can be stored at ambient temperature (15 °C to 30 °C) until the expiration date without affecting component performance.

## Sample and Reagent Volumes

The reagent volumes in the protocol differ based on the starting sample volume. Tables with all of the sample and reagent volumes are included in the Appendix. To make the protocol easy to use, the following icons will be used throughout the protocol to indicate the correct reagent volume for each starting sample volume.

- Green square (■) for **30 mL** sample volume
- Blue circle (●) for **40 mL** sample volume
- Red triangle (▲) for **50 mL** sample volume

For example, ■ 250  $\mu$ L indicates that at this particular step, 250  $\mu$ L would be added if your starting volume was 30 mL.

Please note that these symbols and colors are used to denote a different starting volume in other nRichDX Revolution Kits and their Instructions for Use (IFU). Please ensure you're using the correct IFU for the kit used.

## **General Precautions**

- Ensure you are using the latest published version of this Instructions for Use (IFU) document. The document version is shown on the title page. The latest version of the IFU is found at <https://www.nrichdx.com/>
- Laboratory gloves and other PPE, as well as standard laboratory procedures are recommended when handling chemicals and potentially infectious materials.
- Perform all steps at ambient temperature (15 °C - 30 °C) unless otherwise noted.
- If you observe a precipitate in the Revolution cfDNA Lysis Buffer, incubate the Lysis Buffer at 37 °C until the precipitate dissolves. This can occur if storage temperatures are too low.
- If you observe a precipitate in the Revolution cfDNA Surfactant, incubate the Surfactant at 37 °C until the precipitate dissolves. This can happen if storage temperatures are too low.
- Before use, thoroughly mix the Revolution Antifoaming Agent by vortexing at high speed for 10 seconds. As it is a suspension, complete mixing is essential for consistent performance. Due to its high viscosity, exercise caution and dispense slowly when pipetting the Antifoaming Agent.
- Vortex the Revolution cfDNA Magnetic Beads to fully resuspend them immediately before use.
- Blood samples should be collected in K2EDTA tubes or Streck Cell-Free DNA BCT tubes.
- Cell-free DNA is stable for 24 hours in K2EDTA plasma tubes stored at 2 °C to 10 °C.
- Blood samples collected in Streck Cell-Free DNA BCT tubes remain stable for up to 14 days due to the formaldehyde-free preservative in the tubes.
- For urine samples, use fresh, preferably first-void urine, stored at 2 °C to 10 °C and spun down to cell-free status within 24 hours of collection to avoid increases in genomic DNA (gDNA) and microbial growth.
- If it is not possible to process urine samples immediately after collection, cfDNA urine preservative should be added. Samples collected in the preservative should preferentially be stored and centrifuged per manufacturer recommendations.
- Adequate Capture Tube caps are included in the kit to ensure a new cap is used for each capping step in the protocol. To prevent cross-contamination do not reuse caps.

# Procedure

## 1. Protease Preparation

- 1.1. Transfer 12.5 mL Protease Buffer (PB) to the bottle of Protease Powder (PP).
- 1.2. Cap the bottle and gently invert 8 to 10 times to dissolve the powder.
- 1.3. Place the rehydrated protease solution on ice until use.

**NOTE:** Unused rehydrated protease can be stored at 2 °C to 10 °C for up to three weeks until needed for use. Rehydrated protease older than three weeks may exhibit insufficient protease activity and should be discarded and replaced with freshly prepared protease solution.

## 2. Sample Preparation

### For Plasma Samples

- 2.1. Centrifuge the blood samples at 2,000 x g for 10 minutes at 2 °C to 10 °C.
- 2.2. Transfer the plasma to a new centrifuge tube.
- 2.3. Centrifuge the plasma samples at 16,000 x g for 10 minutes at 2 °C to 10 °C.

**NOTE:** Alternatively, the plasma can be centrifuged at 6,000 x g for 30 minutes to remove any residual blood and cell debris.

- 2.4. Transfer the cell-free supernatants into fresh tubes.
- 2.5. All samples must be equilibrated to one of the starting sample volumes (e.g., 30 mL, 40 mL, 50 mL) based on the following criteria:
  - Samples > 20 mL\* < 30 mL — bring the volume to ■ 30 mL with PBS pH 7.5
  - Samples < 40 mL — bring the volume to ● 40 mL with PBS pH 7.5
  - Samples < 50 mL — bring the volume to ▲ 50 mL with PBS pH 7.5
- 2.6. Process samples immediately or store on ice until use.

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\* For plasma sample volumes of 1 mL - 20 mL, nRichDX recommends the *Revolution cfDNA Max 20 Kit* (PN 408000)

### For Urine Samples

- 2.7. Centrifuge the urine samples at 16,000 x g for 10 minutes at 2 °C to 10 °C.
- 2.8. Transfer the cell-free supernatants into fresh tubes.
- 2.9. All samples must be equilibrated to one of the starting sample volumes (e.g., 30, 40, 50 mL) based on the following criteria:
  - Samples > 20 mL\* < 30 mL — bring the volume to ■ 30 mL with PBS pH 7.5
  - Samples 31 mL < 40 mL bring the volume to ● 40 mL with PBS pH 7.5
  - Samples 41 mL < 50 mL bring the volume to ▲ 50 mL with PBS pH 7.5
- 2.10. Process samples immediately or store on ice until use.

## 3. nRicher Cartridge Sample Tube Preparation

- 3.1. Label the nRicher Cartridge elements (both the Sample Tube and Capture Tube) with a sample identifier; use one Sample Tube per sample.
- 3.2. Place the Sample Tube(s) into the Cartridge Rack



**NOTE:** The Sample Tubes should be left in the rack for the entirety of the extraction.

## 4. Lysis and Bead Binding



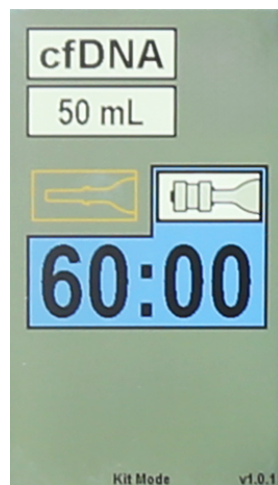
**Figure 11** - GUI for cfDNA bead binding on the Revolution Plus

\*For urine sample volumes of 1 mL - 20 mL, nRichDX recommends the *Revolution cfDNA Max 20 Kit* (PN 408000)



- 4.1. Add cell-free plasma/urine to the labeled Sample Tube.
- 4.2. Add ■ 1.5 mL, ● 2.0 mL, or ▲ 2.5 mL Surfactant Solution to each Sample Tube.
- 4.3. Add ■ 4.8 mL, ● 6.4 mL, or ▲ 8 mL Lysis Buffer to each Sample Tube.
- 4.4. Add ■ 480  $\mu$ L, ● 640  $\mu$ L, or ▲ 800  $\mu$ L Protease Solution to each Sample Tube.
- 4.5. Add ■ 36 mL, ● 48 mL, or ▲ 60 mL Binding Buffer to each Sample Tube.
- 4.6. Vortex the Anti-Foaming Agent for 20 seconds at high speed. Add **20  $\mu$ L** Antifoaming Agent to each sample tube.
- 4.7. Resuspend the Revolution cfDNA Magnetic Beads by vortexing at medium speed for 10 seconds.
- 4.8. Add **300  $\mu$ L** Magnetic Beads to each Sample Tube.
- 4.9. Attach the Capture Tube to its companion Sample Tube and then transfer the Cartridge Rack containing the assembled nRicher Cartridges to the Revolution Processor using the holes positioned on the rack as shown in Figures 9 and 10, on page 8).
- 4.10. Choose the  icon under “cfDNA” and adjust the sample volume accordingly on the Revolution Plus’s Graphical User Interface (GUI). Initiate the process by pressing the  icon.
- 4.11. When the Revolution Plus Processor stops and the GUI illuminates orange, remove the Cartridge Rack containing the nRicher Cartridges from the Revolution Plus and place it on a level surface.

[Continued]

## 5. Bead Capture



**Figure 12** - GUI for cfDNA bead capture on the Revolution Plus

- 5.1. Snap a Mag Capsule onto the Capture Tube of each nRicher Cartridge (Figure 3, page 7), and then place the Cartridge Rack into the Revolution Plus Processor using the holes positioned on the rack to securely attach the Cartridge Rack to the Revolution Plus Processor (Figures 9 and 10, page 8).
- 5.2. Choose the  icon under “cfDNA” and adjust the sample volume accordingly on the Revolution Plus’s GUI. Initiate the procedure by pressing the  icon.
- 5.3. When the Revolution Plus’s motion stops and the GUI illuminates blue, remove the Cartridge Rack from the processor and place the rack on a level surface; let the rack sit for 1 minute to allow all of the liquid to drain from the nRicher Cartridge’s Capture Tube into the Sample Tube.
- 5.4. Remove the Mag Capsule and Capture Tube from each Sample Tube by twisting the Mag Capsule counterclockwise (Figure 3, page 7).  
**IMPORTANT:** Ensure the Mag Capsule remains attached to the Capture Tube for this step.
- 5.5. Gently orient the Mag Capsule and Capture Tube so that the open end of the Capture Tube is facing upward; then press the Mag Capsule down toward the benchtop to release the Capture Tube from the Mag Capsule as shown in Figure 4, page 7. Place the released Capture Tube(s) in a non-magnetic microvial rack.

- 5.6. Discard the liquid remaining in each Sample Tube along with the Sample Tube itself in biohazardous waste.

## 6. Bead Washing

- 6.1. Add 1 mL Wash Solution to each Capture Tube.
- 6.2. Firmly seal each Capture Tube with a new cap and vortex for 10 seconds at medium speed. **IMPORTANT:** Ensure the cap is pressed firmly into the capture tube prior to vortexing to prevent leakage.
- 6.3. Inspect Capture Tube(s) to ensure beads are fully resuspended; if not, then vortex for an additional 10 seconds or until the beads are fully resuspended (no bead clumping is observed).
- 6.4. Centrifuge the Capture Tube(s) for 1 second in a microcentrifuge to collect all remaining liquid from the cap to the bottom of the tube.
- 6.5. Place the Capture Tube(s) into the Mag Rack (Figure 5, page 7) for 2 minutes.
- 6.6. Inspect the Capture Tube(s) to ensure that the supernatant is clear before proceeding to the next step; if the supernatant is not clear, leave the Capture Tube(s) on the Mag Rack for an additional 1 minute.
- 6.7. Remove the cap from each Capture Tube and carefully aspirate and dispose of the supernatant from each tube while the Capture Tube(s) remain in the Mag Rack. **CAUTION:** Be careful not to disturb the bead line and to use a sterile pipette tip for each sample.
- 6.8. Transfer the Capture Tube(s) from the Mag Rack to a non-magnetic microvial rack.
- 6.9. Repeat steps 6.1 to 6.7 for a second wash with Wash Solution.
- 6.10. Add 1 mL 100% ethanol rinse to each Capture Tube.
- 6.11. Recap the Capture Tube(s) and vortex at medium speed for 10 seconds.
- 6.12. Centrifuge the Capture Tube(s) for 1 second in a microcentrifuge to collect all remaining liquid from the cap to the bottom of the Capture Tube.
- 6.13. Place the Capture Tube(s) in the Mag Rack for 2 minutes.
- 6.14. Inspect the Capture Tube(s) to ensure that the supernatant is clear before

proceeding to the next step; if the supernatant is not clear, leave the Capture Tube(s) on the Mag Rack for 1 minute.

- 6.15. Remove the cap from each Capture Tube. Carefully aspirate and dispose of the supernatant from each Capture Tube (using a sterile pipette tip for each sample) while the Capture Tube(s) remain in the Mag Rack.
- 6.16. Transfer the Capture Tube(s) to a non-magnetic microvial rack.
- 6.17. Add 1 mL of 80% ethanol rinse to each Capture Tube.
- 6.18. Recap the Capture Tube(s) and vortex at medium speed for 10 seconds.
- 6.19. Centrifuge the Capture Tube(s) for 1 second in a microcentrifuge to collect all remaining liquid from the cap to the bottom of the Capture Tube.
- 6.20. Place the Capture Tube(s) in the Mag Rack for 2 minutes.
- 6.21. Inspect the Capture Tube(s) to ensure that the supernatant is clear before proceeding to the next step; if the supernatant is not clear, leave the Capture Tube(s) on the Mag Rack for 1 minute.
- 6.22. Remove the cap from each Capture Tube. Carefully aspirate and dispose of the supernatant from each Capture Tube (using a sterile pipette tip for each sample) while the Capture Tube(s) remain in the Mag Rack.
- 6.23. After step 6.22, tap the Mag Rack on the bench 5 times to collect all remaining ethanol to the bottom of the Capture Tube. Carefully aspirate any remaining ethanol from the walls and bottom of the Capture Tube to ensure that the drying time in step 6.25 effectively removes any remaining ethanol.
- 6.24. Quick spin the Capture Tube(s) for 1 second in a microcentrifuge to collect all the beads to the bottom of the Capture Tube.
- 6.25. **BEAD DRYING:** Dry the Capture Tube(s) in a 45 °C heat block for 45 minutes. After drying is complete, transfer the Capture Tube(s) to a nonmagnetic microvial tube rack.

**NOTE:** It is important that the magnetic bead pellet is completely dry and free of ethanol before proceeding. If the beads are not dry enough, place the capture tubes in the 45°C heat block for 1 to 2 more minutes; however, do not overdry the magnetic pellet which may cause the pellet to be difficult to resuspend and lower overall cfDNA yield.



## 7. Elution

- 7.1. **FOR PLASMA:** Add 75  $\mu$ L Elution Buffer to each Capture Tube, and recap.  
**FOR URINE:** Add 50  $\mu$ L Elution Buffer to each Capture Tube, and recap.

**NOTE:** Elution buffer volume may range from 75  $\mu$ L to 100  $\mu$ L for plasma and 50  $\mu$ L to 100  $\mu$ L for urine. The cfDNA concentration will decrease accordingly when eluting in larger volumes.

- 7.2. Briefly vortex the Capture Tube(s) at medium to high speed for 5 to 10 seconds.
- 7.3. Make sure the magnetic beads are completely resuspended in the elution buffer; if the beads are not completely resuspended, or if bead clumping is observed, vortex for another 5 to 10 seconds.
- 7.4. Vortex the Capture Tube(s) on a vortex shaker for 5 minutes at medium to high speed.
- 7.5. Centrifuge the Capture Tube(s) for 1 second in a microcentrifuge to collect all remaining liquid from the cap to the bottom of the tube.
- 7.6. Place the Capture Tube(s) into the Mag Rack for 2 minutes.
- 7.7. Inspect the Capture Tube(s) to ensure that the supernatant is clear before proceeding to the next step; if the supernatant is not clear, leave the Capture Tube(s) on the Mag Rack for another 2 minutes.
- 7.8. Transfer the eluate into a clean, properly labeled tube or plate, making sure not to disrupt the magnetic bead pellet while collecting the eluate (*you may transfer the eluate to any preferred tube or plate suitable for nucleic acid, the elution volume, and your downstream method; for example, a microvial tube, PCR tube, or 96-well plate*).
- 7.9. Discard the Capture Tube(s) containing magnetic beads as biohazardous waste.
- 7.10. Store the eluate at -25 °C to -15 °C for short-term storage (a few days), or at -80 °C for longer-term storage until ready to thaw and use for downstream analysis.

**[End of the nRichDX Revolution Plus cfDNA extraction protocol]**

## Troubleshooting

Observation	Possible cause	Recommended action
Lower yield than expected	The Revolution cfDNA Magnetic Beads were not properly stored	Store the Revolution cfDNA Magnetic Beads at the temperature indicated on the label and package insert. Do not freeze the beads.
	An insufficient amount of Revolution cfDNA Magnetic Beads was added	Immediately before use, vortex the tube containing the magnetic beads thoroughly until fully resuspended.
	The Revolution cfDNA Magnetic Beads are not optimally dried	Drying times may vary depending on the amount of beads used and the environment. Lower volumes of beads require less time to dry. Airflow and humidity in the immediate environment may shorten or lengthen the optimal bead drying time.
		Overdried beads will stick to the wall of the tube and be difficult to resuspend. Gently scrape the beads off the plastic wall using a pipette tip.
		Underdried beads may carry ethanol into the eluate and negatively impact downstream applications. Dry beads slightly longer (1-minute intervals) and make note of the optimal drying time for the specific volume.
	Nucleases	Nuclease contamination will result in a lower yield of intact cfDNA or ctDNA. Ensure reagents, pipette tips and plasticware in direct contact with the sample are free of undesired nucleases. Use nuclease free barrier filter tips and filtered hood to minimize presence of airborne nucleases.
	Magnetic bead clumping is observed	Vortex the tube containing the Magnetic Beads until they are fully resuspended
	The sample contains lower levels of cfDNA than expected	Increase the starting sample volume.
Magnetic bead carryover	Loose beads present in the eluate or inadvertently transferred	Be sure to leave the Capture Tube(s) on the Mag Rack when removing the eluate containing the cfDNA.
		If beads are carried over into the new tube, place the tube on the Mag Rack again, wait for the beads to pellet and then transfer the sample to another tube.

Observation	Possible cause	Recommended action
Fluid leaking from the nRicher Cartridge	Capture Tube not connected correctly to sample tube	Unscrew Capture Tube from Sample Tube. Reattach the Capture Tube to the Sample Tube using the following steps. Lower the Capture Tube opening over the Sample Tube opening. Apply medium pressure to the connection point until a click is heard or sensing the Capture Tube is attached. If the Capture Tube doesn't click, then while applying this pressure, rotate the Capture Tube slightly clockwise and counterclockwise until the click is heard or sense the tube is attached.
Variations in cfDNA yield from donor to donor	Variation in amount of circulating cfDNA. Levels of cfDNA in circulation can range widely depending on the donor.	For samples containing low levels of cfDNA, increase the starting sample volume.

## **Technical Support**

For additional questions, please contact nRichDX's technical support services at [technicalsupport@nrichdx.com](mailto:technicalsupport@nrichdx.com)

## **Reference Materials**

Additional information about the Revolution Plus Processor and operating the instrument in Kit Mode or Research Mode are available in the Revolution Plus User Manual and the separate Revolution Plus Research Mode User Manual (note that operating the Revolution Plus Processor in Research Mode requires a Research Mode Key, PN 200901).

More information at [www.nrichdx.com](http://www.nrichdx.com), or from your nRichDX representative.

## Warnings and Precautions

### For In Vitro Diagnostic Use

Users should wear personal protective equipment as required by local laboratory procedures when performing an isolation, including a lab coat, protective eyewear, and disposable nitrile gloves (or equivalent). Please refer to the relevant safety data sheets (SDSs) for more information.

Discard all used materials as biohazardous waste according to local regulations.

**CAUTION:** The Revolution Lysis Buffer contains guanidinium thiocyanate, which when combined with bleach, forms highly reactive compounds.



**CAUTION: DO NOT directly add bleach or acidic solutions to the isolation waste.**

Clean up all spills with appropriate laboratory-grade detergent and water. Any spills that contain potentially infectious materials should be cleaned first with laboratory detergent and water followed by 1% (v/v) sodium hypochlorite.

If any of the reagent bottles or containers are damaged and leaking fluids, wear gloves and protective eyewear when discarding the bottles.

### Revolution cfDNA Surfactant

**Hazard pictograms:**

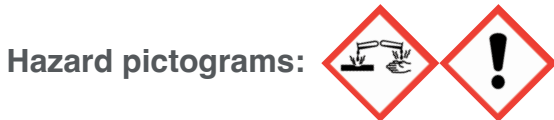


**Signal word:** DANGER

### **Hazard and precautionary statements:**

H315: Causes skin irritation; H318: Causes serious eye damage; P264: Wear protective gloves / protective clothing / eye protection / face protection; P332 + P313: If skin irritation occurs: Get medical advice/attention; P302 + P352: IF ON SKIN - Wash with plenty of soap and water; P305 + P351 + P338: IF IN EYES - Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing; P333 + P313: If skin irritation or rash occurs, get medical advice/attention; P310: Immediately call a POISON CENTER or doctor / physician.

### Revolution cfDNA Lysis Buffer



**Signal word:** DANGER

**Hazard and precautionary statements:**

H302 + H312 + H332: Harmful if swallowed, in contact with skin or inhaled; H314: Causes severe skin burns and eye damage; H412: Harmful to aquatic life with long lasting effects; P261: Avoid breathing dust / fumes / gas / mist / vapors / spray; P264: Wash skin thoroughly after handling; P270: Do not eat, drink or smoke when using this product; P271: Use only outdoors or in well-ventilated area; P273: Avoid release to the environment; P280: Wear protective gloves / protective clothing / eye protection / face protection; P301 + P312 + P330: IF SWALLOWED - Call a POISON CENTER / doctor if you feel unwell. Rinse mouth; P301 + P330 + P331: IF SWALLOWED - Rinse mouth. Do NOT induce vomiting; P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. rinse skin with water / shower; P304 + P340 + P310: IF INHALED - Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER / doctor; P305 + P351 + P338 + P310: IF IN EYES - Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER / doctor; P363: Wash contaminated clothing before reuse; P405: Store locked up; P501: Dispose of contents / container in an approved waste disposal plant.

### Revolution cfDNA Protease Powder

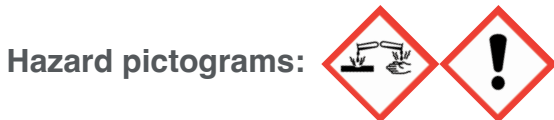


**Signal word:** DANGER

**Hazard and precautionary statements:**

H315: Causes skin irritation; H319: Causes serious eye irritation; H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled; H335: May cause respiratory irritation; P261: Avoid breathing dust / fumes / gas / mist / vapors / 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; P341 + P311: If experiencing respiratory symptoms: Call a POISON CENTER or doctor / physician.

### Revolution cfDNA Protease Buffer

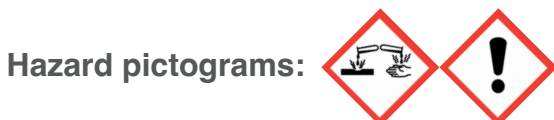


**Signal word:** DANGER

**Hazard and precautionary statements:**

H315: Causes skin irritation; H318: Causes serious eye damage; P280: Wear protective gloves / protective clothing / eye protection / face protection; P264: Wash hands thoroughly after handling; P332 + P313: If skin irritation occurs, get medical advice / attention; P302 + P352: IF ON SKIN - Wash with plenty of soap and water; P305 + P351 + P338: IF IN EYES - Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing; P333 + P313: If skin irritation or rash occurs, get medical advice / attention; P310: Immediately call a POISON CENTER or doctor / physician.

### Revolution cfDNA Binding Buffer



**Signal word:** DANGER

**Hazard and precautionary statements:**

H302: Harmful if swallowed; H314: Causes severe skin burns and eye damage; H412: Harmful to aquatic life with long lasting effects; P260: Do not breathe dust / fume / gas / vapors / spray; P264: Wash hands thoroughly after handling; P270: Do not eat, drink or smoke when using this product; P273: Avoid release to the environment; P280: Wear protective gloves / protective clothing / eye protection / face protection; P301 + P310: IF SWALLOWED - Immediately call a POISON CENTER or doctor / physician; P303 + P361 + P353: IF ON SKIN (or hair) - Remove / take off immediately all contaminated clothing. Rinse skin with water / shower; P 305 + P351 + P338: IF IN EYES - Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing; P310: Immediately call a POISON CENTER or doctor / physician; P330: Rinse mouth.

## Revolution cfDNA Wash Solution

**Hazard pictograms:**



**Signal word:** WARNING

### **Hazard and precautionary statements:**

H302: Harmful if swallowed; H315: Causes skin irritation; H319: Causes serious eye irritation; P264: Wash hands thoroughly after handling; P270: Do not eat, drink or smoke when using this product; P280: Wear protective gloves / protective clothing / eye protection / face protection; P330: Rinse mouth; P332 + P313: If skin irritation occurs, get medical advice / attention; P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P301 + P310: IF SWALLOWED: Immediately call a POISON CENTER or doctor / physician; P302 + P352: IF ON SKIN: Wash with plenty of soap and water.

## Appendix

**Table 1.** Sample and Reagent Volumes

Sample volume (mL)	Surfactant (mL)	Lysis buffer (mL)	Protease solution ( $\mu$ L)	Binding buffer (mL)	Antifoaming Agent ( $\mu$ L)	Magnetic beads ( $\mu$ L)	Total volume* (mL)
30	1.5	4.8	480	36	20	300	73.1
40	2.0	6.4	640	48	20	300	97.4
50	2.5	8.0	800	60	20	300	121.6

**Table 2.** Bead Binding and Bead Capture Incubation Times

Sample volume (mL)	Binding Incubation Time (minutes)	Bead Capture Incubation Time (minutes)
30	30	60
40	30	60
50	30	60

\*Total volume does not include wash solution

Final elution volume is in a range of 75  $\mu$ L - 100  $\mu$ L for plasma and 50  $\mu$ L - 100  $\mu$ L for urine.



## Symbols



In vitro diagnostic medical device



Catalog numbers



Manufacturer



Use-by date



Batch code



Consult instructions for use



Caution



Temperature range

## **Trademarks, Terms, and Warranty Information**

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## The nRichDX Revolution System™

Liquid Biopsy is revolutionizing cancer treatment.  
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