

Instructions for use  
March 2021

Revolution™ cfDNA Max 20 Kit  
Revolution™ cfDNA Max 20XL Kit  
Revolution™ cfDNA Reagent Kit 0.5

Revision F

For in vitro diagnostic use only

**IVD**

**REF**

100131, 100155, 100162



nRichDX, Inc.  
15339 Barranca Pkwy  
Irvine, CA 92618 USA  
+1 949-341-1980

**nRich**<sup>®</sup>**DX**  
DRIVING DIAGNOSTIC EXCELLENCE

# Table of Contents

Intended Use . . . . .	3
Summary and Explanation . . . . .	3
Principles and Procedures . . . . .	4
Materials Provided . . . . .	5
Materials Required but Not Provided . . . . .	7
Revolution nRicher Cartridge Usage and Handling . . . . .	8
Reagent Storing and Handling . . . . .	9
Sample and Reagent Volumes . . . . .	9
General Procedures . . . . .	10
Procedure	
Protease Preparation . . . . .	10
Sample Preparation . . . . .	10
nRichDX Cartridge Preparation . . . . .	11
Protease Treatment . . . . .	12
Bead Binding . . . . .	12
Bead Capture . . . . .	13
Bead Washing . . . . .	13
Elution . . . . .	15
Troubleshooting . . . . .	16
Technical Support . . . . .	17
Warnings and Precautions . . . . .	18
Appendix . . . . .	22
Symbols . . . . .	23
Trademarks and Warranty Information . . . . .	24

## Intended Use

The Revolution cfDNA Max 20 Kit, Revolution cfDNA Max 20XL Kit, and Revolution cfDNA Reagent Kit 0.5 are intended to extract cell-free DNA (cfDNA) from human plasma and urine.

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

These kits are intended for **in vitro diagnostic use only**.

## 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 with minimal cross-contamination between the samples.

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, which enables the users to safely handle potentially infectious samples.

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 -25°C to -15°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.

## **Bead binding**

Revolution cfDNA Magnetic Beads and Revolution cfDNA Binding Buffer are combined with the sample in the Revolution nRicher Cartridge, placed into the Revolution 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, which is again placed into the Processor and incubated to capture the beads in the Microvial Tube portion of the nRicher Cartridge.

## **Bead washing**

The Microvial Tube is removed from the Revolution nRicher Cartridge and washed twice with Revolution cfDNA Wash Solution and then rinsed twice with 80% ethanol using the Revolution Mag Rack to capture the magnetic beads after each wash or 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

## **Revolution cfDNA Max 20 Kit, 100131**

- Revolution cfDNA Surfactant, 7mL, 10007
- Revolution cfDNA Lysis Buffer, 22mL, 10011<sup>†</sup>
- Revolution cfDNA Protease Powder, 40mg, 10012<sup>‡</sup>
- Revolution cfDNA Protease Buffer, 2mL, 10056
- Revolution cfDNA Binding Buffer, 160mL, 10018<sup>†</sup>
- Revolution cfDNA Magnetic Beads, 1.4mL, 10028
- Revolution cfDNA Wash Solution, 54mL, 10084
- Revolution cfDNA Elution Buffer, 3.5mL, 10030
- Revolution nRicher Cartridges, 3 packs of 8 cartridges, 100111
- Instructions for Use (online)

## **Revolution cfDNA Max 20XL Kit, 100155**

- Revolution cfDNA Surfactant, 7mL, 10007
- Revolution cfDNA Lysis Buffer, 22mL, 10011<sup>†</sup>
- Revolution cfDNA Protease Powder, 40mg, 10012<sup>‡</sup>
- Revolution cfDNA Protease Buffer, 2mL, 10056
- Revolution cfDNA Binding Buffer, 160mL, 10018<sup>†</sup>
- Revolution cfDNA Magnetic Beads, 1.4mL, 10028
- Revolution cfDNA Wash Solution, 54mL, 10084
- Revolution cfDNA Elution Buffer, 3.5mL, 10030
- Revolution nRicher Cartridges, 1 pack of 8 cartridges, 100111
- Instructions for Use (online)

---

<sup>†</sup> Contains guanidinium thiocyanate. Do not combine with disinfectants that contain bleach. See page 18 for more information.

<sup>‡</sup> Store at -25°C to -15°C upon receipt

## Materials Provided (continued)

### Revolution cfDNA Reagent Kit 0.5, 100162

- Revolution cfDNA Surfactant 0.5, 3.5mL, 10096
- Revolution cfDNA Lysis Buffer 0.5, 11mL, 10099<sup>†</sup>
- Revolution cfDNA Protease Powder 0.5, 20mg, 100157<sup>‡</sup>
- Revolution cfDNA Protease Buffer 0.5, 1mL, 100158
- Revolution cfDNA Binding Buffer 0.5, 80mL, 10097<sup>†</sup>
- Revolution cfDNA Magnetic Beads 0.5, 0.7mL, 10095
- Revolution cfDNA Wash Solution 0.5, 27mL, 100100
- Revolution cfDNA Elution Buffer 0.5, 1.3mL, 10098
- Instructions for Use (online)

---

<sup>†</sup> Contains guanidinium thiocyanate. Do not combine with disinfectants that contain bleach. See page 18 for more information.

<sup>‡</sup> Store at -25°C to -15°C upon receipt

# Materials Required but Not Provided

Always wear personal protective equipment, such as a lab coat, protective eyewear, and disposable gloves when working with chemicals. Consult the appropriate Safety Data Sheets (SDSs; available from the product supplier) for more information on safe handling and use.

## Revolution equipment

- Revolution Processor, 10081
- Revolution Cartridge Rack, 100305
- Revolution Mag Capsules, 10080
- Revolution Mag Rack, 10082

## Additional materials and reagents

- Pipettors<sup>†</sup>, pipet tips<sup>‡</sup> and serological pipettes
- Microcentrifuge<sup>†</sup>
- Non-magnetic microvial rack
- Aspirator or general laboratory vacuum system
- Vortex instrument with 2mL microvial tube adaptor
- 80% ethanol

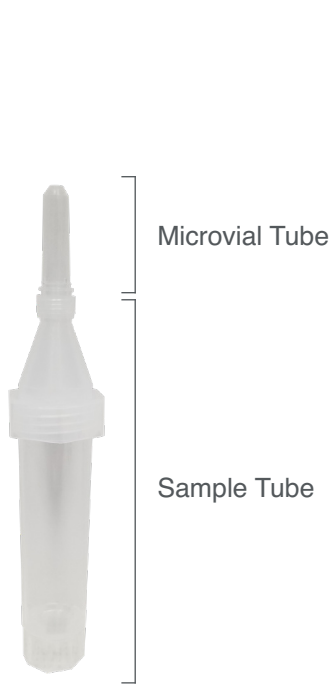
---

<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. However, if using a vacuum for aspiration steps, tips without aerosol barriers should be used.

# nRicher Cartridge Usage and Handling

The nRicher Cartridge combines simplicity with foundational technology to deliver unprecedented cfDNA extraction.



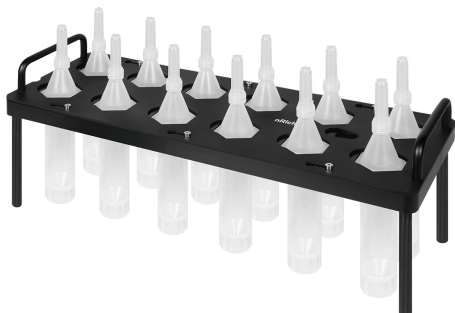
**Figure 1**  
nRicher Cartridge



**Figure 2**  
Removing Microvial Tube



**Figure 3**  
Mag Capsule positioned  
on nRicher Cartridge



**Figure 4**  
nRicher Cartridge components shown in the  
Cartridge Rack; the Microvial Tube is removed and  
placed in a separate microvial rack when accessing  
the Sample Tube portion of the nRicher Cartridge



**Figure 5**  
The Cartridge Rack is  
inserted into the  
Processor



## Reagent Storage and Handling

The Revolution cfDNA kits are shipped at room temperature. **Upon arrival, store the components below at the indicated storage temperatures:**

- Revolution cfDNA Protease Powder should be stored at -25°C to -15°C and can be used until the expiration date without affecting protease performance.
- 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 **5 mL** sample volume
- Blue circle (●) for **10 mL** sample volume
- Tan diamond (◆) for **15 mL** sample volume
- Red triangle (▲) for **20 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 5 mL.

# General Precautions

- Perform all steps at ambient temperature (15°C to 30°C) unless otherwise noted.
- When mixing samples, avoid creating bubbles. If bubbles appear, centrifuge for 1 second to get rid of any bubbles.
- Incubate Revolution cfDNA Lysis Buffer at 37°C until the precipitate dissolves. This can happen if storage temperatures are too low.
- 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.
- Use fresh 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.
- Cell-free urine can be stored at 2°C to 10°C for up to 3 days without degradation.

## 1. Protease Preparation

- 1.1. Transfer Protease Buffer to the bottle of Protease Powder.
- 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 until needed for re-use.**

Reagent Kit	Protease Buffer to Transfer (mL)
Max 20 or Max 20XL	2
Reagent Kit 0.5	1

## 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., 5, 10, 15, 20 mL) based on the following criteria:
  - Samples < 5 mL — bring the volume to ■ 5 mL with PBS pH 7.5
  - Samples < 10 mL — bring the volume to ● 10 mL with PBS pH 7.5
  - Samples < 15 mL — bring the volume to ◆ 15 mL with PBS pH 7.5
  - Samples < 20 mL — bring the volume to ▲ 20 mL with PBS pH 7.5
- 2.6. Process samples immediately or store on ice until use.

#### 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., 5, 10, 15, 20 mL) based on the following criteria:
  - Samples < 5 mL — bring the volume to ■ 5 mL with PBS pH 7.5
  - Samples < 10 mL — bring the volume to ● 10 mL with PBS pH 7.5
  - Samples < 15 mL — bring the volume to ◆ 15 mL with PBS pH 7.5
  - Samples < 20 mL — bring the volume to ▲ 20 mL with PBS pH 7.5
- 2.10. Process samples immediately or store on ice until use.

## 3. nRicher Cartridge Preparation

- 3.1. Label the nRicher Cartridge elements (both Sample Tube and Microvial Tube; see Figure 1, page 8) with a sample identifier; use one nRicher Cartridge per sample.
- 3.2. Place the nRicher Cartridge(s) into the Cartridge Rack.
- 3.3. Remove the Microvial Tube(s) (see Figure 2, page 8), and place the tube(s) into a separate non-magnetic microvial rack.

## 4. Protease Treatment

- 4.1. Add cell-free plasma/urine to the labeled nRicher Cartridge(s).
- 4.2. Add ■ 250  $\mu\text{L}$ , ● 500  $\mu\text{L}$ , ◆ 750  $\mu\text{L}$ , or ▲ 1000  $\mu\text{L}$  Surfactant Solution to each Sample Tube.
- 4.3. Add ■ 800  $\mu\text{L}$ , ● 1600  $\mu\text{L}$ , ◆ 2400  $\mu\text{L}$ , or ▲ 3200  $\mu\text{L}$  Lysis Buffer to each Sample Tube.
- 4.4. Add ■ 80  $\mu\text{L}$ , ● 160  $\mu\text{L}$ , ◆ 240  $\mu\text{L}$ , or ▲ 320  $\mu\text{L}$  Protease Solution to each Sample Tube.
- 4.5. Close the nRicher Cartridge(s) by attaching the Microvial Tube. **CAUTION: Do not over tighten.**
- 4.6. Place the Cartridge Rack containing up to 12 nRicher Cartridges into the Revolution Processor (see Figure 5, page 8).
- 4.7. Start the Processor and incubate at room temperature (15°C to 30°C) at 10 rpm for 1 minute.
- 4.8. When the Processor stops, remove the Cartridge Rack from the device, place the rack on a level surface, and incubate for another 20 minutes at room temperature.

## 5. Bead Binding

- 5.1. Remove the Microvial Tube from each nRicher Cartridge and place it into a separate microvial rack.
- 5.2. Add ■ 6 mL, ● 12 mL, ◆ 18 mL, or ▲ 24 mL Binding Buffer to each Sample Tube.
- 5.3. Resuspend the Revolution cfDNA Magnetic Beads by vortexing at medium speed for 10 seconds.
- 5.4. Add ■ 50  $\mu\text{L}$ , ● 100  $\mu\text{L}$ , ◆ 150  $\mu\text{L}$ , or ▲ 150  $\mu\text{L}$  Magnetic Beads to each Sample Tube.
- 5.5. Attach the Microvial Tube to its companion nRicher Cartridge, and then transfer the Cartridge Rack containing the nRicher Cartridges to the Revolution Processor.
- 5.6. Start the Processor and incubate at room temperature (15°C to 30°C) at 30 rpm

for ■ 30, ● 30, ◆ 45, or ▲ 45 minutes.

- 5.7. When the Processor stops, remove the Cartridge Rack from the device, and place the rack on a level surface.

## 6. Bead Capture

- 6.1. Snap a Mag Capsule onto each nRicher Capsule (see Figure 3, page 8), and then place the Cartridge Rack into the Revolution Processor.
- 6.2. Start the Processor and incubate at room temperature (15°C to 30°C) at 30 rpm for ■ 15, ● 15, ◆ 45, or ▲ 45 minutes.
- 6.3. When the Processor stops, remove the Cartridge Rack from the device, 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 Microvial Tube into the Sample Tube.
- 6.4. Remove the Mag Capsule and Microvial Tube from each nRicher Cartridge by twisting the Mag Capsule counterclockwise.
- 6.5. Orient the Mag Capsule and Microvial Tube so that the rounded bottom of the Microvial Tube is facing downward; then press the Mag Capsule down toward the benchtop to release the microvial tube.
- 6.6. Transfer the Microvial Tube(s) to a non-magnetic microvial rack. **NOTE: Discard the liquid remaining in each Sample Tube along with the Sample Tube itself in biohazardous waste.**

## 7. Bead Washing

- 7.1. Label Microvial Tube Caps to correspond with the sample identifier on each nRicher Capsule.
- 7.2. Add 1 mL Wash Solution to each Microvial Tube.
- 7.3. Seal each Microvial Tube with its corresponding cap and vortex for 10 seconds at medium speed. **NOTE: Inspect Microvial Tube(s) to ensure beads are fully resuspended; if not, then vortex for an additional 10 seconds or until the beads are fully resuspended.**
- 7.4. Centrifuge the Microvial Tube(s) for 1 second in a microcentrifuge to collect

- all remaining liquid from the cap to the bottom of the tube.
- 7.5. Place the Microvial Tube(s) into the Mag Rack for 2 minutes. **NOTE: Inspect the Microvial Tube(s) to ensure that the supernatant is clear before proceeding to the next step; if the supernatant is not clear, leave the Microvial Tube(s) on the Mag Rack for an additional 1 minute.**
  - 7.6. Remove the Microvial Tube Cap from each Microvial Tube and aspirate the supernatant from each Microvial Tube while the Microvial Tube(s) remain in the Mag Rack. **CAUTION: Be careful not to disturb the bead line.**
  - 7.7. Transfer the Microvial Tube(s) from the Mag Rack to a non-magnetic microvial rack.
  - 7.8. Repeat steps 7.2 to 7.7 for a second wash with Wash Solution.
  - 7.9. Add 1 mL 80% ethanol rinse to each Microvial Tube.
  - 7.10. Recap the Microvial Tube(s) and vortex at medium speed for 10 seconds.
  - 7.11. Centrifuge the Microvial Tube(s) for 1 second in a microcentrifuge to collect all remaining liquid from the cap to the bottom of the microvial.
  - 7.12. Place the Microvial Tube(s) in the Mag Rack for 2 minutes. **NOTE: Inspect the Microvial Tube(s) to ensure that the supernatant is clear before proceeding to the next step; if the supernatant is not clear, leave the Microvial Tube(s) on the Mag Rack for 1 minute.**
  - 7.13. Remove the Microvial Tube Cap from each Microvial Tube and aspirate the supernatant from each Microvial Tube while the Microvial Tube(s) remain in the Mag Rack.
  - 7.14. Transfer the Microvial Tube(s) to a non-magnetic microvial rack.
  - 7.15. Repeat rinse steps 7.9 to 7.13 for a second ethanol rinse. **NOTES: After step 7.13, tap the Mag Rack on the bench 5 times to collect all remaining ethanol to the bottom of the Microvial Tube. Carefully aspirate any remaining ethanol from the walls and bottom of the microvial to ensure that the drying time in step 7.16 effectively removes any remaining ethanol.**
  - 7.16. Leave the Microvial Tube(s) in the Mag Rack to dry the magnetic bead pellet for 16 to 20 minutes at room temperature (15°C to 30°C). After drying is complete, transfer the Microvial Tube(s) to a non-magnetic microvial rack. **NOTE: It is important that the magnetic bead pellet is completely dry before proceeding. If the beads slide down the wall of the tube after the tube has been removed from the Mag Rack, the pellet is not sufficiently dry. If the beads are not dry enough, return the microvial to the Mag Rack and dry for an additional 3 to 5 minutes.**

## 8. Elution

- 8.1. Add 50  $\mu\text{L}$  Elution Buffer to each Microvial Tube, and recap. **NOTE: 25  $\mu\text{L}$  and 100  $\mu\text{L}$  elution volumes can be used, although the cfDNA concentration will decrease accordingly when eluting in 100  $\mu\text{L}$ .**
- 8.2. Briefly vortex the Microvial Tube(s) at medium to high speed for 5 to 10 seconds. **NOTE: Make sure the magnetic beads are completely resuspended in the elution buffer; if the beads are not completely resuspended, vortex for another 5 to 10 seconds.**
- 8.3. Vortex the Microvial Tube(s) on a vortex shaker for 5 minutes at medium to high speed.
- 8.4. Centrifuge the Microvial Tube(s) for 1 second in a microcentrifuge to collect all remaining liquid from the cap to the bottom of the tube.
- 8.5. Place the Microvial Tube(s) into the Mag Rack for 2 minutes. **NOTE: Inspect the Microvial Tube(s) to ensure that the supernatant is clear before proceeding to the next step; if the supernatant is not clear, leave the Microvial Tube(s) on the Mag Rack for another 2 minutes.**
- 8.6. Transfer the eluate into a clean, properly labeled PCR tube, making sure not to disrupt the magnetic bead pellet while collecting the eluate. **NOTE: Discard the Microvial Tube(s) containing magnetic beads as biohazardous waste.**
- 8.7. Store the eluate at  $-25^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  until ready for testing.

## 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 15°C to 30°C. 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 re-suspend. 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.
The sample contains low levels of cfDNA	Increase the starting sample volume.		
Magnetic bead carryover	Loose beads present in the eluate or inadvertently transferred	Be sure to leave the Microvial 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.	
Variations in cfDNA yield from donor to donor	Variation in amount of circulating cfDNA. Levels of cfDNA in circulation can range from 1 to 100 ng/mL of plasma or serum depending on the donor.	For samples containing low levels of cfDNA, increase the starting sample volume.	



## Technical Support

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

## 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

**Hazard pictograms:**



**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

**Hazard pictograms:**

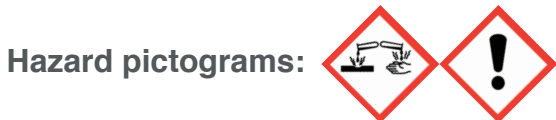


**Signal word:** DANGER

### **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

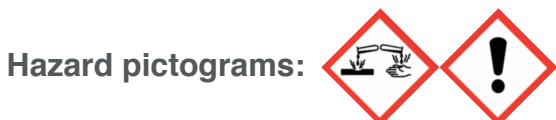


**Signal word:** DANGER

#### **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 ( $\mu$ L)	Lysis buffer ( $\mu$ L)	Protease solution ( $\mu$ L)	Binding buffer (mL)	Magnetic beads ( $\mu$ L)	Total volume (mL)
5	250	800	80	6	50	12.2
10	500	1600	160	12	100	24.4
15	750	2400	240	18	150	36.5
20	1000	3200	320	24	150	48.7

Table 2. Bead Binding and Bead Capture Incubation Times

Sample volume (mL)	Binding Incubation Time (minutes)	Bead Capture Incubation Time (minutes)
5	30	15
10	30	15
15	45	45
20	45	45

## Symbols



In vitro diagnostic medical device



Catalog numbers



Manufacturer



Use-by date



Batch code



Consult instructions for use



Caution

Trademarks: nRichDX® and Revolution™ are trademarks of nRichDX, Inc.  
Liquid Biopsy is revolutionizing cancer treatment. nRichDX empowers that revolution.

nRichDX products are covered by one or more of the following patents: 6,190,469; 10,329,554; 10,585,101; 10,927,366; D886,320; D890,362; Canadian patent number 3,016,895; European patent number 001720118; Japanese patent number 1674486; Korean patent number 30-1062125; Australian patent number 201916131; with other patents pending internationally.

Use of this product indicates the agreement of purchaser or user of this kit to the following terms:

1. This product can only be used in accordance with the protocols and procedures provided in this document using the components in this kit. nRichDX, Inc. grants no license under its intellectual property to employ or integrate the components of their kits with any components not included in the kit except as indicated in the protocols and procedures described herein.
2. nRichDX, Inc. does not warranty that this kit or the use of this kit does not infringe upon the rights of third-party entities.
3. This kit and the components therein are only licensed for one-time use; the kit or components cannot be reused, refurbished or resold.
4. nRichDX disclaims any licenses, expressed or implied, other than the licenses that are expressly stated.
5. The purchaser and the user of this kit agree to not act or permit anyone else to act in any way that might lead to or enable any of the prohibited acts indicated above. nRichDX may enforce the prohibitions of this Limited License Agreement in Court and shall work to recover all investigative and Court costs, including attorney fees, in any action that nRichDX takes to enforce this Limited License Agreement or any intellectual property rights that relate to this kit and/or components of the kit.

© 2021 nRichDX, Inc.



## Revision History

Revision	Date	Description
A	17 September 2019	New document
B	13 November 2019	Edited Bead Binding section 6, step 6.3. Removed the de-gassing step and replaced with the following text, "When the Processor stops, remove the Cartridge Rack from the device, 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 Microvial Tube into the Sample Tube.
C	7 February 2020	Changed Max 10 Kit name to Max 20 and Max 20 Kit name to Max 20XL. In principals of the procedure replaced "denature" with "digest". Updated kit component part numbers. Clarified sample preparation instructions for plasma and urine samples (section 3). Changed bead volume for 15mL sample from 100uL to 150uL.
D	14 July 2020	Grammatical changes. In addition, Process changes include: Pg. 4 from "DNA" to "Magnetic Beads", Pg. 10 from "for one hour" to "until the precipitate dissolves". Inserted table for 1.1 Protease Prep (pg. 10). Switched order of "Step 3. Sample Prep" to "Step 2. Sample Prep" and "Step 2. Cartridge Prep" to "Step 3. Cartridge Prep" (pg. 10-11) in order to match process order in lab. Updated 4.1 to alleviate prior confusing sentence (pg. 12). Update 7.5 to "an additional 1 minute" (pg. 14). Changed bottom of pg. 16 from "Elution Solution" to "Elution Buffer". Updated table 1 so it flows via process by moving Binding Buffer column in front of Magnetic Bead column (pg. 22). Added sentence to a new last page of document: "Liquid Biopsy is revolutionizing cancer treatment. nRichDX empowers that revolution".
E	05 October 2020	Added instructions "After drying is complete, transfer the Microvial Tube(s) to a non-magnetic microvial rack." to step 7.16 (pg. 14) to move microvial tubes to a non-magnetic rack after drying.
F	March 2021	Updated Revolution cfDNA Magnetic Beads storage condition. Updated fill volume in Revolution cfDNA Elution Buffer description. Updated cartridge rack and processor images on page 8. Revised troubleshooting section. Added patent information.

## The nRichDX Revolution System™

Liquid Biopsy is revolutionizing cancer treatment.  
nRichDX empowers that revolution.™