Instructions for Use

Revolution cfTNA Max 20 Kit™
Revolution cfTNA Max 20 Reagent Kit™

Version B

For in vitro diagnostic use only

IVD

REF 408000, 408500

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Intended Use

The Revolution cfTNA Max 20 Kit and Revolution cfTNA Max 20 Reagent Kit are intended to extract cell-free TNA (Total Nucleic Acid) from human plasma and urine.

The kits are intended to be used by physicians 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 cfTNA kits employ well-characterized technology to extract cfTNA which contains cfDNA and cfRNA from 1 mL to 20 mL of plasma or urine samples. The kit procedures are designed so users can process multiple samples simultaneously.

cfTNA extraction from plasma or urine samples >20 mL should use the Revolution cfTNA Max 50 Kit (PN 405500, sold separately).

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

For isolation of cfRNA only, an optional DNase Treatment step may be performed on the eluted cfTNA at the end of the procedure.

Principles of the Procedure

Each Revolution cfTNA kit procedure includes the following steps:

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

Protease Treatment

Enzymes and other proteins are digested in the nRicher Cartridge's Sample Tube (see Figure 1 on page 7 for a diagram of the 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, ensuring that beads are not lost during the procedure.

Bead Binding

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

Bead Capture

The Revolution Mag Capsule is attached to the nRicher Cartridge's Capture Tube. The Cartridges with the attached Mag Capsules are placed into the Revolution Plus Processor and incubated to capture the beads in the Capture Tube section of the nRicher Cartridge.

Bead Washing

The Capture Tube is removed from the nRicher Cartridge and washed twice with Revolution cfTNA Wash Solution and then rinsed once with 100% ethanol and once with 70% ethanol using the Revolution Mag Rack to capture the magnetic beads after each wash or rinse. The beads are then dried.

Elution

The cfTNA is eluted from the beads with Revolution cfTNA 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 ambient temperature.

Revolution cfTNA Max 20 Kit, (# 402500)

- Revolution cfTNA Surfactant, 7 mL, 433000
- Revolution cfTNA Lysis Buffer, 22 mL, 435000†
- Revolution cfTNA Protease Powder, 50 mg, 441000‡
- Revolution cfTNA Protease Buffer, 2.8 mL, 439000
- Revolution cfTNA Binding Buffer, 160 mL, 429000†
- Revolution cfTNA Antifoaming Agent, 500 µL, 410080
- Revolution cfTNA Max 20 Magnetic Beads (Bead 1), 700 µL, 443000‡
- Revolution cfTNA Max 20 Magnetic Beads (Bead 2), 700 µL, 444000
- Revolution cfTNA Wash Solution, 54 mL, 431000
- Revolution cfTNA Elution Buffer, 3.5 mL, 437000
- Revolution nRicher Cartridge 50 mL Sample Tubes, 3 packs of 8 Sample Tubes, 450008
- Revolution nRicher Cartridge Capture Tubes, 3 packs of 8 Capture Tubes, 450004
- Revolution Capture Tube Caps, Qty. 200, 400063
- Instructions for Use (This document. The most current version is available at www.nrichdx.com)

Revolution cfTNA Max 20 Reagent Kit, (# 401500)

- Revolution cfTNA Surfactant, 7 mL, 433000
- Revolution cfTNA Lysis Buffer, 22 mL, 435000†
- Revolution cfTNA Protease Powder, 50 mg, 441000‡
- Revolution cfTNA Protease Buffer, 2.8 mL, 439000
- Revolution cfTNA Binding Buffer, 160 mL, 429000†
- Revolution cfTNA Antifoaming Agent, 500 µL, 410080
- Revolution cfTNA Max 20 Magnetic Beads (Bead 1), 700 µL, 443000‡
- Revolution cfTNA Max 20 Magnetic Beads (Bead 2), 700 µL, 444000
- Revolution cfTNA Wash Solution, 54 mL, 431000
- Revolution cfTNA Elution Buffer, 3.5 mL, 437000
- Instructions for Use (This document. The most current version is available at www.nrichdx.com)

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

‡ 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, 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, 200917
- Revolution Cartridge Rack, 200600
- Revolution Mag Capsules, 200700
- Revolution Mag Rack, 200800
- Revolution Plus Processor, 2000PLUS

Additional Materials and Reagents (Molecular Biology Grade)

- 70% Ethanol
- 96% - 100% Ethanol
- Beta-Mercaptoethanol (BME)
- Isopropanol (IPA)
- Centrifuge and Microcentrifuge†
- 50 mL Conical Tube
- 500 mL Sterile Bottle
- Non-magnetic microvial rack*
- Phosphate Buffered Saline (PBS) pH 7.5
- Pipettors†, pipette tips‡, and serological pipettes
- 37 °C Incubator
- 60 °C Heating Block
- Vortex instrument with 2 mL microvial tube adaptor*
- DNAse - if DNase digestion of eluted cfTNA is desired to further isolate cfRNA

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

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

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

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 (haptic) sensation as shown in Figure 2. To remove the Capture 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/counter-clockwise 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.

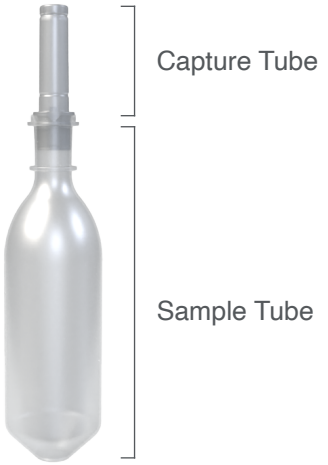

Figure 1 - nRicher Cartridge
The nRicher Cartridge is comprised of the connected Sample Tube and Capture Tube as shown

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

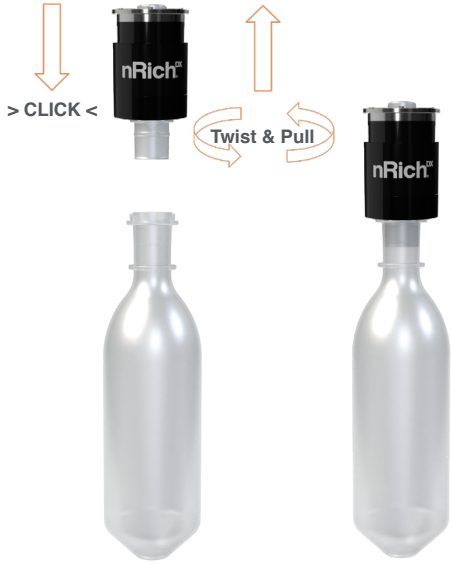

Figure 3 - Mag Capsule
Joining or separating the Mag Capsule and Capture Tube from the Sample Tube

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)

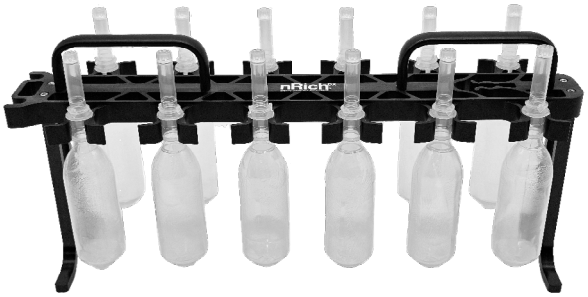

Figure 6 - Cartridge Rack with Cartridges
nRicher Cartridges shown in the Cartridge Rack; the Capture Tube is removed and placed in a separate rack when accessing the Sample Tube portion of the nRicher Cartridge.

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 Storage and Handling

The Revolution cfTNA kits are shipped at room temperature.

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

- Revolution cfTNA Protease Powder should be stored in a freezer at -25 °C to -15 °C and can be used until the kit expiration date without affecting protease performance.
- Revolution cfTNA Magnetic Beads 1 and 2 should be stored at the temperature indicated on the label and the package insert.
- All other kit components can be stored at indoor 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 µL indicates that at this particular step, 250 µL 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.
- If you observe a precipitate in the Revolution cfTNA Lysis Buffer, incubate the Lysis Buffer

at 37 °C until the precipitate dissolves. This can occur if storage temperatures are low.

- If you observe a precipitate in the Revolution cfTNA 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 Antifoaming Agent by vortexing at high speed for 20 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 cfTNA Magnetic Beads to fully resuspend them immediately before use.
- Blood samples should be collected in K2EDTA tubes or Streck Cell-Free TNA BCT tubes.
- 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, cfTNA urine preservative should be added to the sample. nRichDX recommends nRichDX RNA Urine Preservative, PN 427000, sold separately. 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 2.5 mL of 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. Lysis Solution Preparation

2.1. According to Table 1 below, transfer an aliquot of Lysis Buffer to an appropriately sized conical tube and label it “Lysis Solution.”

2.2. Add BME to Lysis Solution conical tube according to instructions in Table 1.

Table 1. Volumes of Lysis Buffer and BME for processing

	Lysis Buffer (**mL**)				BME (***µL***)			
Number of Samples	■ 5 mL	● 10 mL	◆ 15 mL	▲ 20 mL	■ 5 mL	● 10 mL	◆ 15 mL	▲ 20 mL
1	1.0 mL	2.0 mL	3.0 mL	4.0 mL	55 µL	110 µL	165 µL	220 µL
2	1.8	4.0	5.5	7.5	100	200	300	400
3	2.6	5.5	8.0	10.5	150	300	450	600
4	3.4	7.0	10.5	13.5	200	400	600	800
5	4.2	8.5	13.0	17.0	250	480	720	960
6	5.0	10.0	15.5	20.0	300	570	860	1150
7	5.8	12.0	18.0	23.5	340	660	1000	1300
8	6.6	13.5	20.5	26.5	380	750	1130	1500
9	7.4	15.0	23.0	30.0	420	850	1300	1700
10	8.2	16.5	25.5	33.0	470	940	1400	1900
11	9.0	18.5	28.0	36.0	520	1000	1600	2100
12	9.8	20.5	30.5	39.5	570	1100	1700	2250

NOTE: Lysis Buffer units in **mL** and BME units in ***µL***

2.3. Cap the tube and invert 8 – 10 times to combine the Lysis Buffer and BME into a uniform solution.

3. Binding Solution Preparation

3.1. According to Table 2, transfer an aliquot of Binding Buffer to an appropriately sized sterile bottle and label it “Binding Solution.”

3.2. Add IPA to Binding Solution Bottle according to instructions in Table 2. Process samples immediately or store on ice until use.

3.3. Cap the bottle and invert 8 – 10 times to combine the Binding and IPA into a uniform solution.

Table 2. Volumes for Binding Buffer and Isopropanol (IPA) for processing ■ 5 mL, ● 10 mL, ◆ 15 mL, or ▲ 20 mL samples.

	Binding Buffer (**mL**)				IPA (**mL**)			
Number of Samples	■ 5 mL	● 10 mL	◆ 15 mL	**20 mL**	**5 mL**	**10 mL**	**15 mL**	**20 mL**
1	5	10	14	19	2.5	5.0	7.0	9.5
2	9	18	26	35	4.5	9	13	17.5
3	13	26	38	51	6.5	13	19	25.5
4	17	34	50	67	8.5	17	25	33.5
5	21	42	62	83	10.5	21	31	41.5
6	25	50	74	99	12.5	25	37	49.5
7	29	58	86	115	14.5	29	43	57.5
8	33	66	98	131	16.5	33	49	65.5
9	37	74	110	147	18.5	37	55	73.5
10	41	82	122	163	20.5	41	61	81.5
11	45	90	134	179	22.5	45	67	89.5
12	49	98	146	195	24.5	49	73	97.5

4. Sample Preparation

For Plasma Samples

4.1. Centrifuge the blood samples at 2,000 x g for 10 minutes at 2 °C to 10 °C.

4.2. Transfer the plasma to a new centrifuge tube.

4.3. Centrifuge the plasma samples at 1,300 x g for 10 minutes at 2 °C to 10 °C.

4.4. Transfer the cell-free supernatants into fresh tubes.

4.5. 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 > 5 mL < 10 mL — bring the volume to ● 10 mL with PBS pH 7.5
- Samples > 10 mL < 15 mL — bring the volume to ◆ 15 mL with PBS pH 7.5
- Samples > 15 mL < 20 mL — bring the volume to ▲ 20 mL with PBS pH 7.5

4.6. Process samples immediately or store on ice until use.

For Urine Samples

4.7. Centrifuge the urine samples at 16,000 x g for 10 minutes at 2 °C to 10 °C.

4.8. Transfer the cell-free supernatants into fresh tubes.

4.9. 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 > 5 mL < 10 mL — bring the volume to ● 10 mL with PBS pH 7.5
- Samples > 10 mL < 15 mL — bring the volume to ◆ 15 mL with PBS pH 7.5
- Samples > 15 mL < 20 mL — bring the volume to ▲ 20 mL with PBS pH 7.5

4.10. Process samples immediately or store on ice until use.

5. nRicher Cartridge Preparation

5.1. Label the nRicher Cartridge components (both the Sample Tube and Capture Tube; see Figure 1, page 7) with a sample identifier; use one Sample Tube per sample.

5.2. 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.

5.3. Remove the Capture Tube(s) (see Figure 2, page 7), and place the tube(s) into a separate non-magnetic capture tube rack.

NOTE: Capture Tubes will fit in most microvial tube racks.

6. Sample Treatment

6.1. Add cell-free plasma/urine to the labeled Sample Tube.

6.2. Add ■ 80 µL, ● 160 µL, ◆ 240 µL, or ▲ 320 µL Protease Solution to each Sample Tube.

6.3. Add ■ 250 µL, ● 500 µL, ◆ 750 µL, or ▲ 1000 µL Surfactant Solution to each Sample Tube.

6.4. Add ■ 850 µL, ● 1700 µL, ◆ 2600 µL, or ▲ 3400 µL Lysis Buffer (prepared in Section 2) to each Sample Tube.

6.5. Add ■ 6 mL, ● 12 mL, ◆ 18 mL, or ▲ 24 mL Binding Buffer (prepared in Section 3) to each Sample Tube.

6.6. Vortex the Anti-foaming Agent for 20 seconds at high speed. For all sample volumes, add 10 µL Anti-foaming Agent to each sample tube.

6.7. Resuspend the Revolution cfTNA Magnetic Beads 1 and Revolution cfTNA Magnetic Beads 2 by vortexing each tube at medium speed for 30 seconds.

6.8. Prepare a working solution of Beads 1 and 2 by mixing in a 1:1 ratio, **NOTE**: Prepare sufficient working solution for the number of samples to be extracted per sample as follows: ■ 50 µL, ● 100 µL, ◆ 150 µL, or ▲ 150 µL

6.9. Vortex the bead working solution until mixed thoroughly. Add ■ 50 µL, ● 100 µL, ◆ 150 µL, or ▲ 150 µL Magnetic Beads to each Sample Tube.

6.10. Close the Sample Tube(s) by attaching the Capture Tube to its companion Sample Tube. Place the nRicher Cartridge(s) (Sample Tube + Capture Tube connected. See Figure 1, page 7) in the nRicher Cartridge Rack as shown in Figure 6 on page 8.

NOTE: A tip for connecting the Capture Tube and Sample Tube. Position the Capture Tube opening over the Sample Tube opening. Apply continuous medium pressure until a click sound is heard (and haptic feedback is felt) and the Capture Tube is snug on the Sample tube as shown in Figure 2 on page 7.

6.11. Once all samples have been capped invert the Sample Tube Rack 10 times and ensure the beads have successfully mixed with the sample.

6.12. For ■ 5 mL, ● 10 mL, ◆ 15 mL incubate 10 minutes at 37 °C. For ▲ 20 mL incubate 20 minutes at 37 °C. NOTE: If space permits transfer entire Cartridge Rack into incubator. If necessary, remove the Sample Tubes from the Rack and place into the incubator. After incubation, place the Sample Tubes back into Cartridge Rack.

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Please proceed to Step 7 on the next page.]

7. Bead Binding

Figure 11 - GUI for cfTNA bead binding on the Revolution Plus

7.1. After incubation, place the Cartridge Rack containing the assembled nRicher Cartridges into the Revolution Plus Processor as described in Figures 9 and 10 on page 8. Close the processor door.

7.2. Choose the icon under “cfTNA” and select the sample volume using the volume arrow icon on the Revolution Plus’s Graphical User Interface (GUI). Start the process by pressing the icon.

7.3. When the Revolution Plus Processor stops and the GUI illuminates orange, remove the Cartridge Rack from the device (see Figure 10, page 8), and place the rack on a level surface.

8. Bead Capture

Figure 12 - GUI for cfTNA bead capture on the Revolution Plus

8.1. Attach 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 on the rack to securely attach the Cartridge Rack to the Revolution Plus Processor (see Figures 9 and 10, page 8). Close the processor door.

8.2. Choose the  icon under “cfTNA” and select the starting sample volume on the Revolution Plus’s GUI using the  icon. Initiate the procedure by pressing the  icon.

8.3. 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 Cartridge’s Capture Tube into the Sample Tube.

8.4. Remove the Mag Capsule and the Capture Tube from each Sample Tube by twisting the Mag Capsule counterclockwise as shown in Figure 3, page 7). **IMPORTANT:** Ensure the Mag Capsule remains attached to the Capture Tube for this step.

8.5. 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 on page 7. Place the released Capture Tube(s) in a non-magnetic rack.

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

9. Bead Washing

9.1. Add 1 mL Wash Solution to each Capture Tube.

9.2. Firmly seal each Capture Tube with its 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.

9.3. 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 and no bead clumping is observed.

9.4. 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.

9.5. Place the Capture Tube(s) into the Mag Rack (Figure 5, page 7) for 2 minutes.

9.6. 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.

9.7. Remove the cap from each Capture Tube and carefully aspirate all of the supernatant from each tube while the Capture Tube(s) remain in the Mag Rack. Dispose of the supernatant. **CAUTION:** Be careful not to disturb the magnetic bead line on the side of the Capture Tube and use a sterile pipette tip for each sample.

9.8. Transfer the Capture Tube(s) from the Mag Rack to a non-magnetic tube rack

9.9. Repeat steps 9.2 to 9.9 for a second wash with Wash Solution.

9.10. Add 1 mL 96% - 100% ethanol rinse to each Capture Tube.

9.11. Recap the Capture Tube(s) and vortex at medium speed for 10 seconds.

9.12. 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.

9.13. Place the Capture Tube(s) in the Mag Rack for 2 minutes.

9.14. 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.

9.15. 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.

9.16. Transfer the Capture Tube(s) to a non-magnetic Capture Tube rack. (Capture Tubes fit in most standard laboratorymicrovial tube racks)

9.17. Add 1 mL 70% ethanol rinse to each Capture Tube.

9.18. Recap the Capture Tube(s) and vortex at medium speed for 10 seconds.

9.19. 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.

9.20. Place the Capture Tube(s) in the Mag Rack for 2 minutes.

9.21. 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 an additional 1 minute, or until the supernatant is clear.

9.22. Remove the Capture Tube Cap from each Capture Tube and aspirate the supernatant from each Capture Tube while the Capture Tube(s) remain in the Mag Rack.

9.23. After step 9.24, tap the Mag Rack on the bench 5 times to collect all remaining ethanol to the bottom of each Capture Tube in the Mag Rack. Carefully aspirate, using a small volume P10 pipette and sterile tip, any remaining ethanol from the walls and bottom of the Capture Tube.

9.24. For all sample volumes, quick spin the Capture Tube(s) for 1 second in a microcentrifuge to collect all the beads to the bottom of the Capture Tube.

9.25. BEAD DRYING: For ■ 5 mL quick spin in a microcentrifuge followed by drying the Capture Tube(s) in a 37 ˚C heat block for 30 minutes; for ● 10 mL quick spin microcentrifuge followed by drying the Capture Tube(s) in a 37 ˚C heat block for 40 minutes; for ◆ 15 mL and ▲ 20 mL, quick spin microcentrifuge followed by drying the Capture Tube(s) in a 37 ˚C heat block for 50 minutes. After drying is complete, transfer the Capture Tube(s) to a nonmagnetic Capture 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 37 ˚C heat block for an additional 3 to 4 minutes; however, do not overdry the magnetic pellet which may cause the pellet to be difficult to resuspend and lower overall cfTNA yield.

10. Elution

10.1. Add 50 µL Elution Buffer to each Capture Tube, and recap.

NOTE: Elution buffer volume may range from 25 µL to 100 µL. The cfTNA concentration will decrease accordingly when eluting in larger volumes.

10.2. Vortex the Capture Tube(s) at medium to high speed for 5 to 10 seconds.

10.3. 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.

10.4. 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.

10.5. Add the capped Capture Tube(s) to a heating block and incubate for 5 minutes at 60 °C.

10.6. Vortex the Capture Tube(s) on a vortex shaker for 5 minutes at medium to high speed.

10.7. 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.

10.8. Place the Capture Tube(s) into the Mag Rack for 2 minutes.

10.9. Inspect the Capture Tube(s) to ensure that the supernatant (eluate) is clear before proceeding to the next step; if the supernatant is not clear, leave the Capture Tube(s) in the Mag Rack for another 2 minutes.

10.10. Transfer the eluate into a clean, properly labeled tube of your choice (such as a PCR tube), making sure not to disrupt the magnetic bead pellet while collecting the eluate.

10.11. Discard the Capture Tube(s) containing magnetic beads as biohazardous waste.

10.12. 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 cfTNA extraction protocol]

Optional DNase Treatment Step for Removal of cfDNA and Isolation of cfRNA Followed by Recommended Clean-Up Step

NOTE: If isolated cfRNA only is desired, perform DNase treatment per DNase provider's instructions followed by clean-up post DNAase treatment to remove inhibitors and store eluates at -25 °C to -15 °C for short-term storage (a few days), or at -80 °C for longer-term storage.

Troubleshooting

Observation	Possible cause	Recommended action
Lower yield than expected	The Revolution cfTNA Magnetic Beads were not properly stored	Store the Revolution cfTNA Magnetic Beads at the temperature indicated on the label and package insert. Do not freeze the beads.
	An insufficient amount of Revolution cfTNA Magnetic Beads was added	Immediately before use, vortex the tube containing the magnetic beads thoroughly until fully resuspended.
	cfTNA Magnetic Beads were not sufficiently mixed	Immediately before use, vortex the tube containing the magnetic beads thoroughly until fully resuspended. Do not allow beads to settle.
	The Revolution cfTNA 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 cfTNA and/or cfRNA. Ensure reagents, pipette tips and plasticware in direct contact with the sample are free of undesired nucleases. Use nuclease free barrier filter pipet tips and a filtered hood to minimize the presence of airborne nucleases.
	Magnetic bead clumping is observed	Vortex the tube containing the Magnetic Beads until they are fully resuspended
	The sample contains low levels of cfTNA	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 cfTNA.
		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	Capture Tube not connected correctly to sample tube	Disconnect the Capture Tube from the Sample Tube as shown in Figures 2 and 3 on page 7. Reattach the Capture Tube to the Sample Tube using the instructions provided.
Variations in cfTNA yield from donor to donor	Variation in amount of circulating cfDNA and cfRNA depending on the donor.	For samples containing low levels of cfDNA or cfRNA, increase the starting sample volume.

Technical Support

For additional questions, please contact technical support services at **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 Revolution Plus Research Mode User Manual. These materials are available on 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

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 Powder

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

Hazard pictograms: 

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

Hazard pictograms: 

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 3. Sample and Reagent Volumes (per extraction)

Sample volume (mL)	Surfactant (µL)	BME (µL)	Lysis buffer (µL)	Protease solution (µL)	IPA (mL)	Binding buffer (mL)	Antifoaming Agent (µL)	Magnetic beads (µL)	Total volume (mL)
5	250	50	800	80	2	4	10	50	12.2
10	500	100	1600	160	4	8	10	100	24.5
15	750	150	2400	240	6	12	10	150	36.7
20	1000	200	3200	320	8	16	10	150	48.9

*Please note **mL** and **µL** units in the table above.

Table 4. Bead Binding and Bead Capture Incubation Times

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

Final elution volume is in a range of 25 µL - 100 µL; 50 µL is the default recommended elution volume.

Symbols

In vitro diagnostic medical device

Catalog numbers

Manufacturer

Use-by date

Batch code

Consult instructions for use

Caution

Temperature range

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