Instructions for Use

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

Revision D

For in vitro diagnostic use only

IVD

REF 100487, 100499, 100500

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

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POWERING LIQUID BIOPSY

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Intended Use

The Revolution cfTNA Max 20 Kit, Revolution cfTNA Max 20XL Kit, and Revolution cfTNA Reagent Kit are intended to extract cell-free Total Nucleic Acid (cfTNA) 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 cfTNA kits employ well-characterized technology to extract cell-free RNA (cfRNA) and cell-free DNA (cfDNA) from as little as 1 mL up to 20 mL volumes of plasma or urine samples per extraction. 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, which enables the users to safely handle potentially infectious samples.

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 for later use.

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 Revolution nRicher Cartridge.

Bead Binding

Revolution cfTNA Magnetic Beads 1 and 2 are mixed and together with Revolution cfTNA 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 cfTNA 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 cfTNA Wash Solution and then rinsed once with 96%-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, 100499

- Revolution cfTNA Surfactant, 7mL, 100493
- Revolution cfTNA Lysis Buffer, 22mL, 100490†
- Revolution cfTNA Protease Powder, 40mg, 100492‡
- Revolution cfTNA Protease Buffer, 2mL, 100491
- Revolution cfTNA Binding Buffer, 160mL, 100488†
- Revolution cfTNA Magnetic Beads 1, 700µL, 100525‡
- Revolution cfTNA Magnetic Beads 2, 700µL, 100526‡
- Revolution cfTNA Wash Solution, 54mL, 100494
- Revolution cfTNA Elution Buffer, 3.5mL, 100489
- Revolution nRicher Cartridges, 3 packs of 8 cartridges, 100111
- Instructions for Use (online at nrichdx.com)

Revolution cfTNA Max 20XL Kit, 100500

- Revolution cfTNA Surfactant, 7mL, 100493
- Revolution cfTNA Lysis Buffer, 22mL, 100490†
- Revolution cfTNA Protease Powder, 40mg, 100492 [Store Frozen]‡
- Revolution cfTNA Protease Buffer, 2mL, 100491
- Revolution cfTNA Binding Buffer, 160mL, 100488†
- Revolution cfTNA Magnetic Beads 1, 700µL, 100525‡
- Revolution cfTNA Magnetic Beads 2, 700µL, 100525‡
- Revolution cfTNA Wash Solution, 54mL, 100494
- Revolution cfTNA Elution Buffer, 3.5mL, 100489
- Revolution nRicher Cartridges, 1 pack of 8 cartridges, 100111
- Instructions for Use (online at nrichdx.com)

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† Contains guanidinium thiocyanate. Do not combine with disinfectants that contain bleach. See page 22 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.

Materials Provided (continued)

Revolution cfTNA Reagent Kit, 100487

- Revolution cfTNA Surfactant, 7mL, 100493
- Revolution cfTNA Lysis Buffer, 22mL, 100490†
- Revolution cfTNA Protease Powder, 40mg, 100492‡
- Revolution cfTNA Protease Buffer, 2mL, 100491
- Revolution cfTNA Binding Buffer, 160mL, 100488†
- Revolution cfTNA Magnetic Beads 1, 700µL, 100525‡
- Revolution cfTNA Magnetic Beads 2, 700µL, 100526‡
- Revolution cfTNA Wash Solution, 54mL, 100494
- Revolution cfTNA Elution Buffer, 3.5mL, 100489
- Instructions for Use (online at nrichdx.com)

† Contains guanidinium thiocyanate. Do not combine with disinfectants that contain bleach. See page 22 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.

Other Materials Required (Not Included)

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:

- Drip Pan, 100140
- Revolution Cartridge Rack, 100305
- Revolution Mag Capsules, 10080
- Revolution Mag Rack, 10082
- Revolution Processor, 10081

Additional Materials and Reagents

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

†We strongly recommend that instruments are calibrated at regular intervals to ensure that samples are processed consistently and accurately, recommend Myfuge Mini Centrifuge at Thomas Scientific

‡We strongly recommend using pipette tips with aerosol barriers to prevent cross contamination. Recommend Vortex Genie 2 at Scientific Industries, Inc.

nRicher Cartridge Usage and Handling

The nRicher Cartridge combines simplicity with foundational technology to deliver unprecedented cfTNA extraction. See this brief Microvial Tube attachment video for more information on correctly attaching the nRicher Cartridge's Sample Tube and cap.

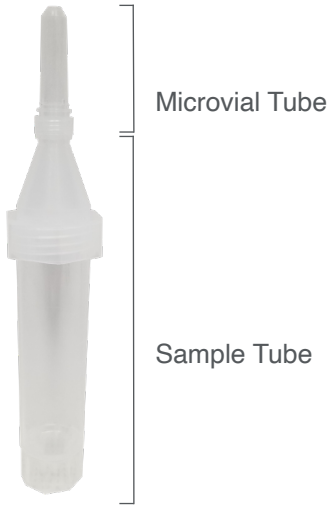


Figure 1
nRicher Cartridge

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

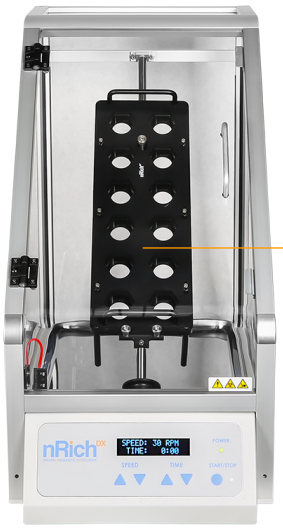


Figure 5
The Cartridge Rack is inserted into the Processor

Reagent Storage and Handling

The Revolution cfDNA kits are shipped at room temperature.

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

- Revolution cfTNA 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 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 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 happen if storage temperatures are too 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.
- Vortex the Revolution cfTNA Magnetic Beads to fully resuspend them immediately before use.
- 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.
- If it is not possible to process urine samples immediately after collection, TNA urine preservative should be added. Samples collected in the preservative should preferentially be stored and centrifuged per manufacturer recommendations.

Procedure

1. Protease Solution Preparation

1.1. Transfer 2mL of 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.

2. Lysis Solution Preparation

2.1. According to Table 1, transfer aliquot of Lysis Buffer to separate 50 mL conical tube. Label 50 mL conical tube as Lysis Solution.

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

Table 1. Volumes for Lysis Buffer and BME for processing ■ 5 mL, ● 10 mL, ◆ 15 mL, or ▲ 20 mL samples.

Number of Samples	Lysis Buffer (mL)				BME (µL)			
	■	●	◆	▲	■	●	◆	▲
1	1.0	2.0	3.0	4.0	55	110	165	220
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	1,150
7	5.8	12.0	18.0	23.5	340	660	1,000	1,300
8	6.6	13.5	20.5	26.5	380	750	1,130	1,500
9	7.4	15.0	23.0	30.0	420	850	1,300	1,700
10	8.2	16.5	25.5	33.0	470	940	1,400	1,900
11	9.0	18.5	28.0	36.0	520	1,000	1,600	2,100
12	9.8	20.5	30.5	39.5	570	1,100	1,700	2,250

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 and BME into a uniform solution.

3. Binding Solution Preparation

3.1. According to Table 2, transfer aliquot of Binding Buffer to 500 mL Sterile Bottle. Label 500 mL Sterile Bottle as 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.

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

Number of Samples	Binding Buffer (mL)				IPA (mL)			
	■	●	◆	▲	■	●	◆	▲
1	5	10	14	19	2.5	5	7	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

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

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 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.

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 < 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

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 < 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

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

5. nRicher Cartridge Preparation

5.1. 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.

5.2. Place the nRicher Cartridge(s) into the Cartridge Rack.
NOTE: The cartridges should be left in the rack for the entirety of the extraction.

5.3. Remove the Microvial Tube(s) (see Figure 2, page 8), and place the tube(s) into a separate non-magnetic microvial rack.

6. Sample Treatment

6.1. Add cell-free plasma/urine to the labeled nRicher Cartridge(s).

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 Solution from Step 2 to each Sample Tube.

6.5. Add ■ 6 mL, ● 12 mL, ◆ 18 mL, or ▲ 24 mL Binding Solution from Step 3

to each Sample Tube.

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

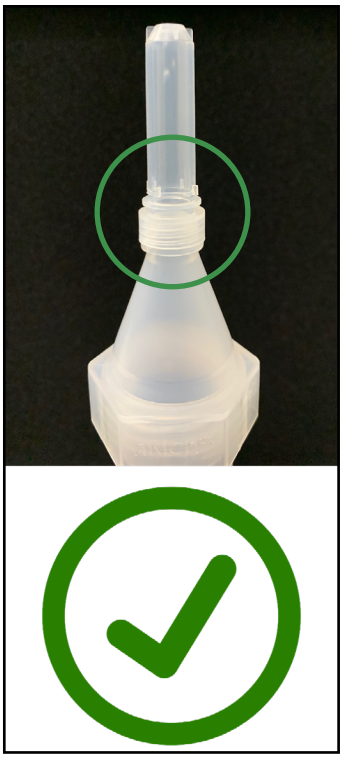
6.7. Prepare a working solution of Beads 1 and 2 by mixing in a 1:1 ratio. Note: Prepare sufficient bead volume for ■ 50 µL, ● 100 µL, ◆ 150 µL, or ▲ 150 µL Magnetic Beads to each Sample Tube.

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

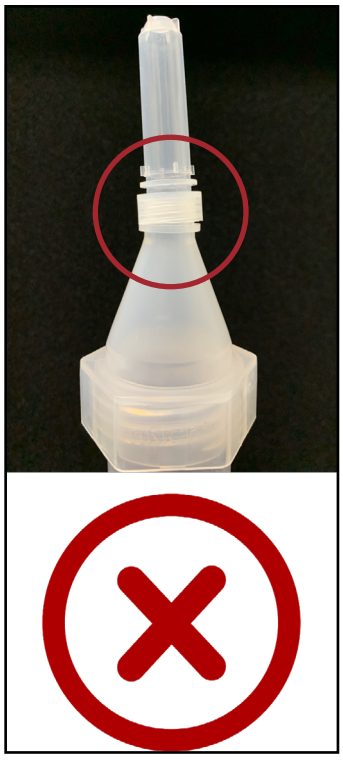
6.9. Close the nRicher Cartridge(s) by attaching the Microvial Tube.
CAUTION: Do not over tighten.

NOTE: A tip for connecting the elution tube and sample tube. Position the microvial tube opening over the sample tube opening. Apply medium pressure at the connection point and rotate the microvial tube clockwise to tighten. Repeat until connection is snug. Finally, ensure the microvial tube is aligned vertically. If not vertical, apply light pressure to the microvial tube in the opposite direction of the tilt. After alignment, check to ensure connection is firm and even as shown below (see short video for details).

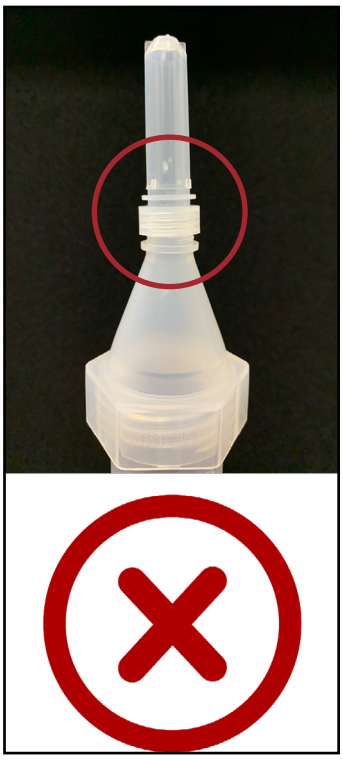
Correct
(snug/tight/even)



Incorrect
(cross-threaded/uneven)



Incorrect
(loose/not snug)



6.10. Place the Cartridge Rack containing up to 12 nRicher Cartridges into the Revolution Processor (see Figure 5, page 8).

6.11. Start the Processor and incubate at room temperature (15°C to 30°C) at 10 rpm for 1 minute.

6.12. When the Processor stops, remove the Cartridge Rack from the device,

6.13. For ■ 5 mL, ● 10 mL, ◆ 15 mL incubate 10 minutes at 37°C. For ▲ 20 mL incubate for 20 minutes at 37°C.

NOTE: If space permits transfer entire Cartridge Rack into incubator. If necessary, remove nRicher Cartridges from Rack and place into incubator. After incubation, place nRicher Cartridges back into Rack.

7. Bead Binding

7.1. After incubation, place Cartridge Rack into device and start the Processor as follows:

7.1.1 For all ■ 5 mL samples place in the processor for 30 minutes at 30 rpm. For all ● 10 mL, ◆ 15 mL, and ▲ 20 mL samples place in the processor for 45 minutes at 30 rpm.

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

8. Bead Capture

8.1. Snap a Mag Capsule onto the Microvial Tube for each nRicher Cartridge as shown in Fig. 3 on p. 8 (avoid opening by twisting - ensure the connection remains tight), and place the Cartridge Rack into the Revolution Processor.

8.2. Start the Processor as follows:

8.2.1. For all ■ 5 mL samples place in the processor for 15 minutes at 30 rpm. For all ● 10 mL, ◆ 15 mL, and ▲ 20 mL samples place in the processor for 30 minutes at 30 rpm

8.3. When the Processor completely stops, remove the Cartridge Rack from the

device. Place the rack on a level surface and let the rack sit for 1 minute to allow all the liquid to drain from the Microvial Tube into the Sample Tube.

8.4. Remove the Mag Capsule and Microvial Tube from each nRicher Cartridge by twisting the Mag Capsule counterclockwise.

8.5. 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.

8.6. Transfer the Microvial Tube(s) to a non-magnetic microvial rack.

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

9. Bead Washing

9.1. Label Microvial Tube Caps to correspond with the sample identifier on each nRicher Capsule.

9.2. Add 1 mL Wash Solution to each Microvial Tube.

9.3. Seal each Microvial Tube with its corresponding cap and vortex for 10 seconds at medium speed.

NOTE: A tip for connecting the cap and microvial tube. Lower the cap plug into the microvial tube opening. Apply light to medium pressure to the connection point. While applying this pressure, rotate the cap plug clockwise to tighten the connection. Repeat until the connection is snug.

9.4. 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.

9.5. 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.

9.6. Place the Microvial Tube(s) into the Mag Rack for 2 minutes.

9.7. 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.

9.8. 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.

NOTE: Be sure supernatant is removed entirely .

9.9. Transfer the Microvial Tube(s) from the Mag Rack to a non-magnetic microvial rack.

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

9.11. Add 1 mL 96-100% ethanol rinse to each Microvial Tube.

9.12. Recap the Microvial Tube(s) and vortex at medium speed for 10 seconds.

9.13. 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.

9.14. Place the Microvial Tube(s) in the Mag Rack for 2 minutes.

9.15. 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.

9.16. 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.

9.17. Transfer the Microvial Tube(s) to a non-magnetic microvial rack.

9.18. Add 1 mL 70% ethanol rinse to each Microvial Tube.

9.19. Recap the Microvial Tube(s) and vortex at medium speed for 10 seconds.

9.20. 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.

9.21. Place the Microvial Tube(s) in the Mag Rack for 2 minutes.

9.22. 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.

9.23. 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.

9.24. Transfer the Microvial Tube(s) to a non-magnetic microvial rack.

9.25. After step 9.24, tap the Mag Rack on the bench 5 times to collect all remaining ethanol to the bottom of the Microvial Tube. Carefully aspirate, using a P10 pipette, any remaining ethanol from the walls and bottom of the Microvial.

9.26. For all sample volumes, pulse centrifuge the microvials for 1 second in a microcentrifuge to collect all the beads to the bottom of the microvial.

BEAD DRYING: For 5mL sample volumes dry the beads for 30 minutes in a 37˚C heat block; for 10mL sample volumes dry the beads for 40 minutes in a 37˚C heat block; for 15mL and 20mL sample volumes dry the beads for 50 minutes in a 37˚C heat block.

CAUTION: It is important that the magnetic bead pellet is completely dry before proceeding. If the beads are not completely dry, place the microvials in a 37˚C heat block for 3 to 4 more 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 Microvial Tube, and recap.
NOTE: 25 µL and 100 µL elution volumes can be used, although the cfTNA concentration will decrease accordingly when eluting in 100 µL.

10.2. Briefly vortex the Microvial 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, vortex for another 5 to 10 seconds.

10.4. 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.

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

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

10.7. 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.

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

10.9. 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.

10.10. Transfer the eluate into a clean, properly labeled PCR tube, making sure not to disrupt the magnetic bead pellet while collecting the eluate.

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

10.12. Store the eluate at -25°C to -15°C until ready for downstream analysis.

Optional DNase Treatment Step for Isolation of cfRNA

NOTE: If isolated cfRNA is desired, perform DNase treatment per DNase provider's instructions and store eluates at -80°C.

[End of nRichDX Revolution cfTNA Extraction Protocol]

Troubleshooting

Observation	Possible cause	Recommended action
Lower yield than expected	The Revolution cfTNA 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 cfTNA Magnetic Beads was added	Ensure that working solution of magnetic beads is prepared in a 1:1 ratio from Revolution cfTNA Magentic Beads 1 and Revolution cfTNA Magentic Beads 2. Ensure that both tubes of beads were vortexed immediately prior to mixing. Do not allow beads to settle.
	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 cfDNA and/or cfRNA. Ensure reagents, pipet 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 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 Microvial 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
Microvial Tube not vertical and/or not snug on Sample Tube	Microvial not connected correctly to sample tube	Unscrew Microvial from sample tube. Reattach Microvial to the sample tube using the following steps. Lower the Microvial tube opening over the sample tube opening. Apply medium pressure to the connection point. While applying this pressure, rotate the microvial tube clockwise to tighten the connection. Repeat until the connection is snug. Finally, ensure the microvial tube is aligned vertically. If not vertical, apply a light pressure to the microvial tube in the opposite direction of the tilt. After alignment, check to ensure connection is snug (see reference photo on page <?>). Also see the Microvial Tube attachment to Sample Tube and cap for reference.
Variations in cfTNA yield from donor to donor	Variation in amount of circulating cfTNA. Levels of cfTNA in circulation can range from 1 to 100 ng/mL of plasma or serum de-pending on the donor.	For samples containing low levels of cfTNA, increase the starting sample volume.

Technical Support

For additional questions, please contact nRichDX technical support at **technicalsupport@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 cfTNA 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 cfTNA 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 cfTNA 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 cfTNA 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 cfTNA 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 cfTNA 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.

Beta-Mercaptoethanol (BME)

Hazard pictograms: 

Signal word: WARNING

Hazard and precautionary statements:
H301: Toxic if swallowed; H302: Harmful if swallowed; H310: Fatal in contact with skin; H311: Toxic in contact with skin; H314: Causes severe skin burns and eye damage; H315: Causes skin irritation; H317: May cause an allergic skin reaction; H318: Causes serious eye damage; H331: Toxic if inhaled; H332: Harmful if inhaled; H336: May cause drowsiness or dizziness; H361: Suspected of damaging fertility or the unborn child; H373: Causes damage to organs through prolonged or repeated exposure; H400: Very toxic to aquatic life; H410: Very toxic to aquatic life with long lasting effects; H411: Toxic to aquatic life with long lasting effects; P203: Obtain, read and follow all safety instructions before use; P260: Do not breathe dust/fume/gas/mist/vapors/spray; P261: Avoid breathing dust/fume/gas/mist/vapors/spray; P262: Do not get in eyes, on skin, or on clothing; P264: Wash hands thoroughly after handling; P264+P265: Do not touch eyes; P270: Do not eat, drink or smoke when using this product; P271: Use only outdoors or in a well-ventilated area; P272: Contaminated work clothing should not be allowed out of the workplace; P273: Avoid release to the environment, P280: Wear protective

gloves/protective clothing/eye protection/face protection/hearing protection; P301+P316: IF SWALLOWED: Get emergency medical help immediately; P301+P317: IF SWALLOWED: Get emergency medical help; P301+P330+P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting; P302+P352: IF ON SKIN: Wash with plenty of water; P302+P361+P354: IF ON SKIN: Take off immediately all contaminated clothing. Immediately rinse with water for several minutes; P304+P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing; P305+P354+P338: IF IN EYES: Immediately rinse with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing; P316: Get emergency medical help immediately; P317: Get emergency medical help; P318: If exposed or concerned, get medical advice; P319: Get medical help if you feel unwell; P332+P317: IF SKIN irritation occurs: Get emergency medical help; P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P361+P364: Take off immediately all contaminated clothing. And wash it before reuse; P362+P364: Take off contaminated clothing. And wash it before reuse; P363: Wash contaminated clothing before reuse; P391: Collect spillage; P403+P233: Store in a well-ventilated place. Keep container tightly closed; P405: Store locked up; and P501: Dispose of contents/container.

Isopropanol (IPA)

Hazard pictograms: 

Signal word: WARNING

Hazard and precautionary statements:

H225: Highly Flammable liquid and vapor; H319: Causes serious eye irritation; H336: May cause drowsiness or dizziness; P210: Keep away from heat, hot surface, sparks, open flames and other ignition sources. No smoking; P233: Keep container tightly closed; P240: Ground/bond container and receiving equipment; P241: Use explosion-proof equipment; P242: Use only non-sparking tools; P243: Take precautionary measures against static discharge; P261: Avoid breathing dust/ fume/gas/mist/vapors/spray; P264+P265: Wash hands thoroughly after handling. Do not touch eyes; P271: Use only outdoors or in a well-ventilated area; P280: Wear protective gloves/ protective clothing/eye protection/face protection/hearing protection; P303+P361+P353: IF ON SKIN (or hair): Take off Immediately all contaminated clothing. Rinse SKIN with water [or shower]; P304+P340: IF INHALED: Remove person to fresh air and keep comfortable for breathing; P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do - continue rinsing; P319: Get medical help if you feel unwell;

P337+P317: If eye irritation persists: Get medical help; P403+P233: Store in a well-ventilated place. Keep container tightly closed; P403+P235: Store in a well-ventilated place. Keep cool; P405: Store locked up; and P501: Dispose of contents/container.

Appendix

Table 1. Sample and Reagent Volumes

Sample volume (mL)	Surfactant (µL)	BME (µL)	Lysis buffer (µL)	Protease solution (µL)	IPA (mL)	Binding buffer (mL)	Magnetic beads (µL)	Total volume (mL)
5	250	46	800	80	2	4	50	12.2
10	500	92	1600	160	4	8	100	24.5
15	750	138	2400	240	6	12	150	36.7
20	1000	184	3200	320	8	16	150	48.9

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	45	30
15	45	30
20	45	30

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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