

Instructions for Use

Revolution CTC Enrichment Kit (Epithelial Origin)[™]

Version A

For Research Use Only. Not for use in diagnostic procedures

RUO

REF

100560



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POWERING LIQUID BIOPSY[®]

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Intended Use

The nRichDX Revolution Circulating Tumor Cells (CTC) Enrichment Kit (Epithelial Origin) is intended to isolate EpCAM-expressing cells from human whole blood.

The kit is intended to be used by researchers and technicians that have received training in molecular biology laboratory techniques.

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

Summary and Explanation

The nRichDX Revolution CTC Enrichment Kit (Epithelial Origin) utilizes Revolution magbead chemistry with anti-EpCAM antibodies using proprietary nRicher Cartridges and the Revolution System Processor to capture intact circulating tumor cells (CTCs) originating from epithelial tissues from whole blood samples.

The Revolution CTC Enrichment Kit (Epithelial Origin) can isolate CTCs from 1 mL to 40 mL of whole blood. The kit procedures are designed so users can process multiple samples simultaneously.

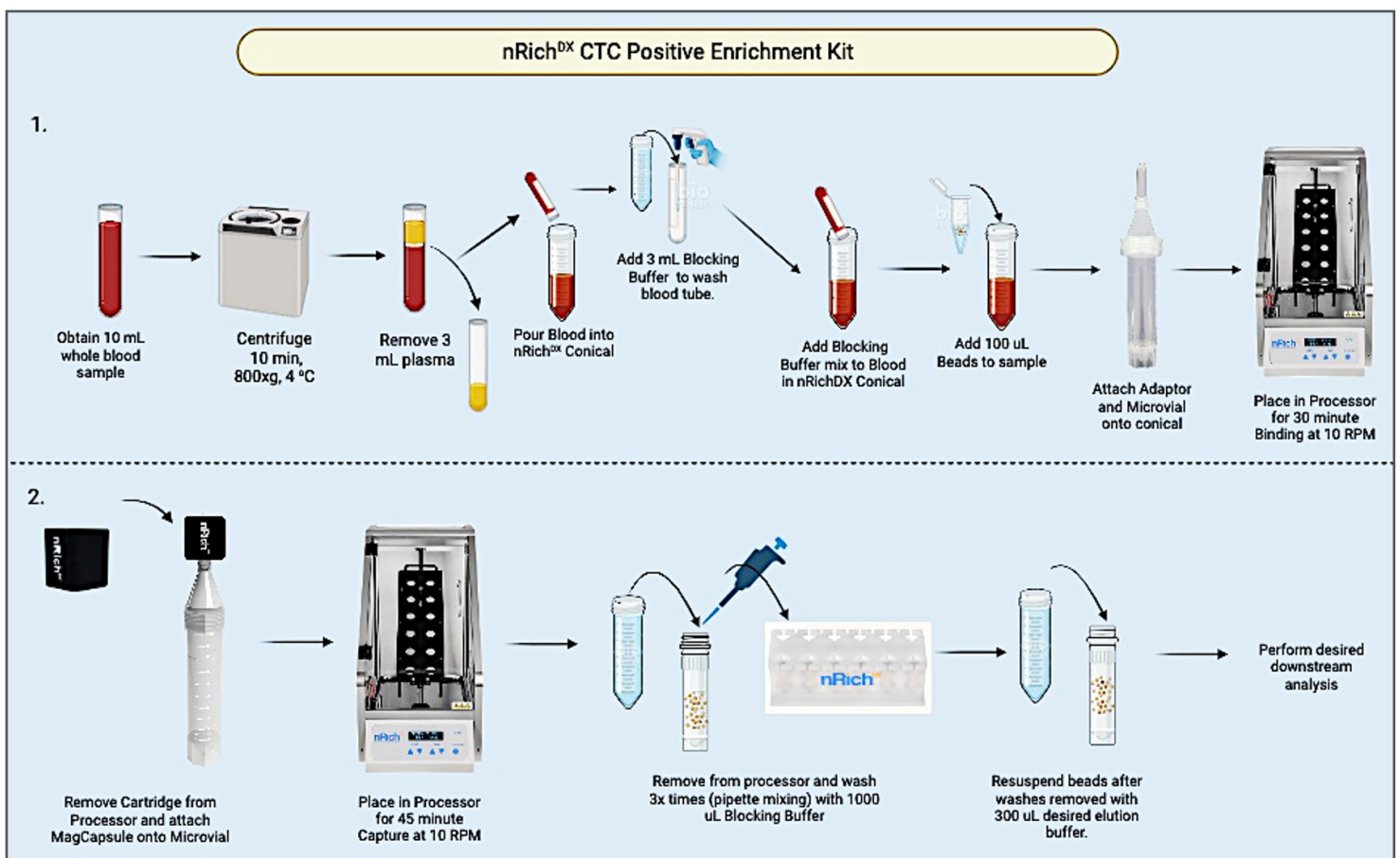
The procedures are designed for minimal user handling, which enables the users to safely handle potentially infectious samples.

The isolated CTCs are ready for use in downstream applications, such as Immunocytochemistry (ICC) and nucleic acid extraction using Revolution cfDNA or cfTNA Kits followed by nucleic acid analysis methods including PCR, real-time PCR (RT-PCR), and Next-Generation Sequencing (NGS). Alternatively, the purified CTCs may be stored for later use.

Principles of the Procedure

Each Revolution CTC Enrichment Kit (Epithelial Origin) procedure includes the following steps as described and shown below:

- Preprocessing of whole blood samples (centrifugation) and addition of Enrichment Buffer
- Sample preparation by addition of nRichDX EpCAM immunomagnetic beads
- Bead binding, capture and washing
- Elution of CTCs from beads



Revolution CTC Enrichment Procedure

Materials Provided

- nRichDX Revolution CTC Enrichment Kit
- nRichDX anti-EpCAM Magnetic Beads, 1 mL,
- nRichDX Enrichment Powder, 100 mg
- nRicher Cartridges with adapters, 2.0 mL microvials, and caps, 10 ea.

Other Materials Required (Not Included)

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

Revolution System Equipment:

- Drip Pan, 100140
- Revolution Cartridge Rack, 10005
- Revolution Mag Capsules, 10080
- Revolution Mag Rack, 10082
- Revolution Processor, 10081

Additional Materials and Reagents^{†‡}

- Phosphate buffered saline (PBS) pH 7.5
- Centrifuge with swing bucket rotor and Microcentrifuge
- Non-magnetic microvial rack
- Pipettors, pipet tips, and serological pipettes
- Vortex instrument

[†]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.

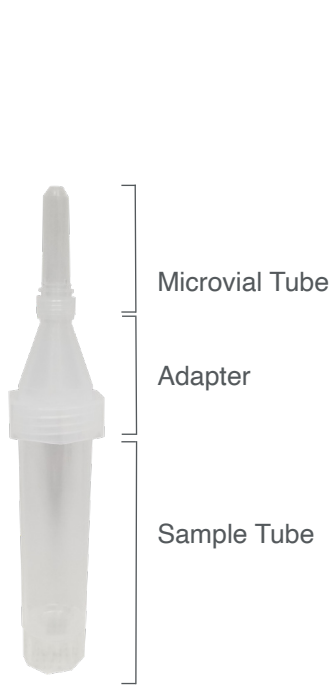


Figure 1
nRicher Cartridge



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

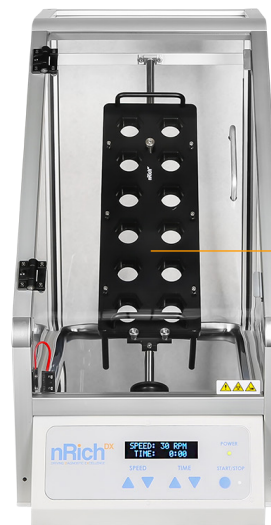


Figure 5
The Cartridge Rack is inserted into the Processor

Reagent Storage and Handling

The Revolution CTC Enrichment Kits are shipped at 2°C to 8°C.

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

- nRichDX anti-EpCAM Magnetic Beads should be stored at 2°C to 8°C and can be used until the kit expiration date.
- nRichDX Enrichment Powder should be stored at 2°C to 8°C .

General Precautions

- Blood samples should be collected in K2EDTA or CellSave tubes.
- Blood samples collected in K2EDTA or CellSave tubes remain stable for up to 48 hours when stored at 2°C to 8°C.
- When handling blood, users should follow all safety regulations and wear personal protective equipment as required.
- Perform all steps at ambient temperature (18°C to 28°C) unless otherwise noted.
- Pulse-vortex the nRichDX EpCAM Beads to fully resuspend them immediately before use.

Procedure

1. Enrichment Buffer Preparation

- 1.1. Add 100 mL of 1X PBS to the bottle containing the Enrichment Powder. Gently agitate to fully dissolve.

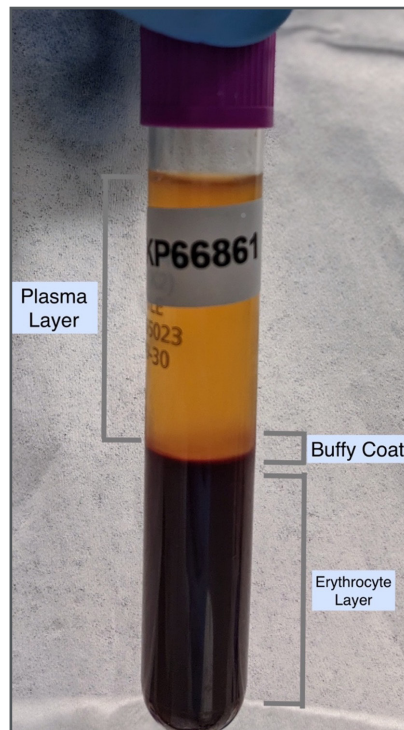
NOTE: Avoid vortexing.

- 1.2. Store at 2°C to 8°C for 30 days or at -25 to -15 °C for 1 year. Avoid multiple freeze-thaw cycles of the Enrichment Buffer.

2. Preprocessing of Whole Blood

- 2.1. Obtain whole blood sample in an appropriate collection tube or container.
- 2.2. Centrifuge samples using a swing bucket rotor with brakes off for 10 minutes at 800xg at 4°C.
- 2.3. Remove 3 mL of plasma from the top layer from each collection tube, or 3 mL of plasma per 10 mL whole blood, making sure to avoid disturbing the buffy coat layer.

NOTE: Plasma obtained during this step can also be used as samples in the Revolution cfDNA Max 20 Kit for isolation of cfDNA and the nRichDX cfTNA Max 20 Kit for isolation of circulating cell-free total nucleic acid or cfRNA.



- 2.4. Transfer the remaining sample into a 50 mL conical tube (provided in kit) by gently pouring the sample down the inner wall of the tube at a slight angle.
- 2.5. To recover any blood sample remaining, add 3 mL Enrichment Buffer to the blood collection tube using a P1000 pipette.
- 2.6. If using a sample collected in a different container, wash with 3 mL of Enrichment Buffer for every 10 mL of whole blood.
- 2.7. Gently pipette up and down using a P1000 pipette tip to resuspend and mix the Enrichment Buffer and remaining blood.
- 2.8. Transfer the remaining blood sample to the 50 mL conical tube(s) from step 2.5 with the P1000 pipette.
- 2.9. If the sample volume in the conical tube after step 2.8 is <10 mL, add Enrichment Buffer to the sample to bring it up to a total volume of 10 mL.
- 2.10. Process samples immediately or store on ice until use.

3. nRicher Cartridge Microvial Preparation

- 3.1. Label the nRicher Microvial Tube with a sample identifier; use one nRicher Adaptor and Microvial Tube per sample.
- 3.2. Place the Microvial Tubes(s) into a separate non-magnetic microvial rack.

4. Sample Preparation

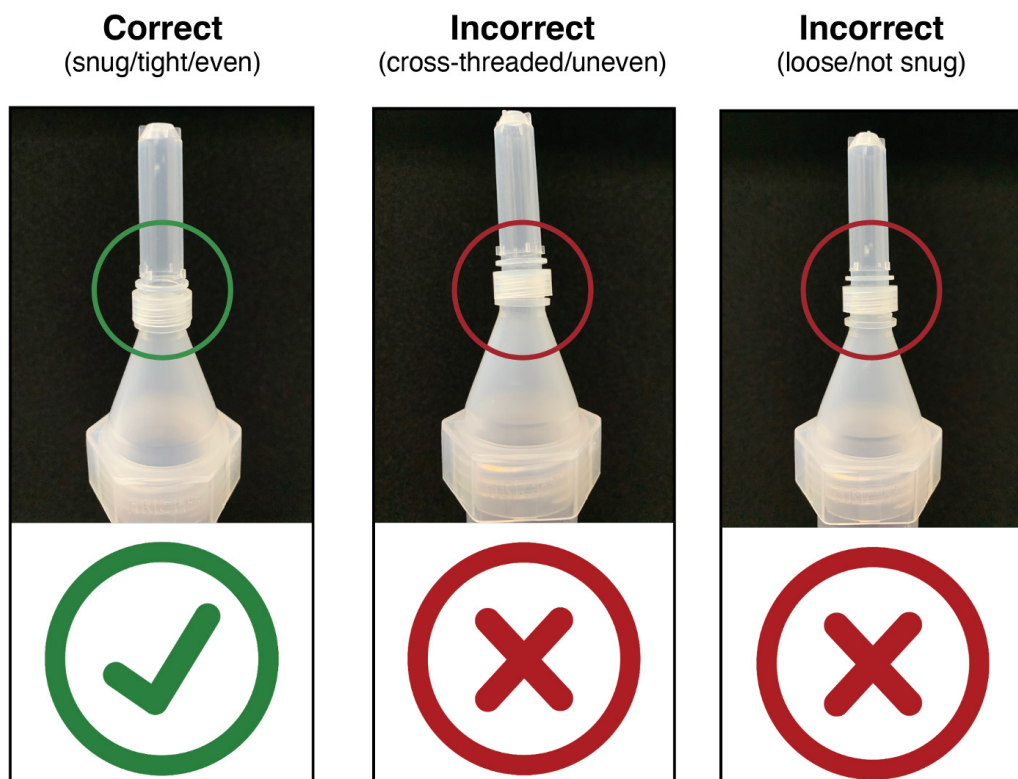
- 4.1. Resuspend the nRichDX EpCAM Beads by pulse vortexing at medium speed 10-15 times. Ensure that beads are homogeneously suspended.
- 4.2. Add 100 μ L nRichDX EpCAM Beads to each Sample Tube if sample volume is \leq 10 mL. If sample volume is between 10 mL and 40 mL, add 250 μ L of the beads (see Table 1).

Table 1: Volumes of beads needed for processing (per sample)

Sample Volume (mL)	Blood Collection Tube(s)	Bead Volume (μ L)
1-10	1	100
>10-40	2-4	250

- 4.3. Attach the nRicher Cartridge Adaptor to each 50 mL conical tube by tightening it in a clockwise direction. Ensure the Adaptor is securely tightened.
- 4.4. Close the nRicher Cartridges by attaching the Microvial Tube.
CAUTION: Do not over tighten.

NOTE: A tip for connecting the microvial tube to the adaptor. Position 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 firm and even as shown below.



- 4.5. Place the nRicher Cartridge(s) in the Cartridge rack.

5. Bead Binding

- 5.1. Place the Cartridge Rack containing up to 12 nRicher Cartridges into the Revolution Processor.
- 5.2. Start the Processor for 30 minutes at 10 RPM.
- 5.3. 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 the Microvial Tube for each nRicher Cartridge, and place the Cartridge Rack into the Revolution Processor.
- 6.2. Start the Processor for 45 minutes at 10 RPM.
- 6.3. When the Processor stops, remove the Cartridge Rack from the device, and place it on a level surface. Let the rack sit for 1 minute to allow all of the liquid to drain from the 2 mL microvial.
- 6.4. Remove the Mag Capsule and 2 mL microvial from the Cartridge by twisting the Mag Capsule counter-clockwise.
- 6.5. Orient the Mag Capsule and 2 mL microvial so the rounded bottom of the microvial is facing downward; then press the Mag Capsule down on the benchtop to snap release the microvial tube.
- 6.6. Centrifuge for 1 second in a microcentrifuge to collect all remaining liquid from the sides of the microvial to the bottom.
- 6.7. Transfer the 2 mL microvial to a non-magnetic microvial rack.
- 6.8. Discard the liquid remaining in the Sample Tube along with the Sample tube itself as biohazardous waste.

7. Bead Washing

- 7.1. Add 1 mL of Enrichment Buffer to each Microvial Tube.
- 7.2. Resuspend the beads using a P1000 pipette by gently pipetting up and down 5-10 times or until no clumps are present.
- 7.3. Cap the Microvial Tube(s) and centrifuge the Microvial Tubes(s) for 1 second in a microcentrifuge to collect all remaining liquid from the sides of the microvial to the bottom of the tube. Make sure that after the centrifugation the beads are still resuspended.
- 7.4. Place Microvial Tube(s) in the Mag Rack for 2 minutes.
- 7.5. When the supernatant is fully clear, use a P1000 pipette to aspirate the supernatant from the microvial while the microvial is on the Mag Rack. Be careful not to disturb the bead pellet.
- 7.6. Transfer the microvial from the Mag Rack to a non-magnetic microvial rack.
- 7.7. Perform steps 7.2 to 7.7 an additional 2 times for a total of 3 washes.

8. Elution

- 8.1. Transfer the 2 mL microvial to a non-magnetic microvial rack .
- 8.2. Add 300-1000 μL of Enrichment Buffer to the microvial tube.
- 8.3. Using a P1000 pipette, gently resuspend the beads containing the target cells by pipetting up and down.
- 8.4. Proceed to downstream analysis or preserve the eluates per your downstream analysis preservation recommendation.

Troubleshooting

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.
Not enough plasma in sample	Sample is not viable for enrichment	Re-collect sample from patient.
No clear whole blood layer separation after centrifugation	Sample is past stable period for storage and handling	Re-collect sample from patient.
	Sample has not been stored in correct conditions	Re-collect sample from patient. Ensure all equipment needed for storage conditions meet minimum requirements.
Whole blood layers are slanted after centrifugation	Fixed rotor was used during sample preprocessing centrifugation	Swing bucket rotor should be used during the centrifugation step of sample-preprocessing to obtain flat layer separation
Variation in amount of beads per sample (e.g. different sizes of bead lines during washes)	Beads not fully resuspended prior to use	Pulse vortex bead vial just prior to use to ensure beads are fully resuspend and are homogenous. Pipetting up and down when aspirating beads will also aid in homogenization.

Technical Support

For additional questions, please contact nRichDX technical support at technicalsupport@nrichdx.com

Warnings and Precautions

For Research Use Only. Not for use in diagnostic procedures.

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.

Anti-EpCAM [VU-1D9] CTC Enrichment Beads

Signal word: None

Hazard and precautionary statements:

P301 + P330: IF SWALLOWED: Rinse mouth; P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Dispose of contents / container in an approved waste disposal plant.

Revolution Enrichment Buffer

Signal word: None

Hazard and precautionary statements:

P301 + P330: IF SWALLOWED: Rinse mouth; P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.

Dispose of contents / container in an approved waste disposal plant.

Symbols



Research Use Only. Not for use in diagnostic procedures.



Catalog numbers



Manufacturer



Use-by date



Batch code



Consult instructions for use



Caution



Temperature range

Trademarks, Terms, and Warranty Information

Trademarks: nRichDX[®], nRicher[™], Revolution Sample Prep System[™], Revolution cfTNA Max 20 Kit[™], Revolution cfTNA Max 20XL Kit[™], Revolution cfTNA Reagent Kit[™], Revolution cfDNA Max 20 Kit[™], Revolution cfDNA Max 20XL Kit[™], Revolution cfDNA Reagent Kit[™], Revolution CTC Enrichment Kit (Epithelial Origin)[™], “Driving Diagnostic Excellence[®],” and “Liquid Biopsy is revolutionizing cancer treatment. nRichDX empowers that revolution.[®],” are trademarks of nRichDX, Inc.

nRichDX products are covered by one or more of the following patents: 10,329,554; 10,585,101; 10,927,366; D886,320; D890,362; Canada patent numbers 3,016,895; 3,042,426; 190733; Europe patent numbers 001720118; 3535058; China patent number ZL2017800688437; Japan patent number 389863; Korea patent numbers 30-1062125; 10-2239349; Australia patent numbers 201916131; 2017355635, with other patents pending internationally.

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

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4. nRichDX disclaims any licenses, expressed or implied, other than the licenses that are expressly stated.
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Revision	Date	Description
A	October 2023	New document. Version A of the Revolution CTC Enrichment Kit (Epithelial Origin) Instructions for Use (IFU).

The nRichDX Revolution System™

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

