Instructions for Use

May 2022

Semi-Automated Workflow on the Eppendorf® ep*Motion*® using:

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

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

RUO

REF 100131, 100155, 100119

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DRIVING DIAGNOSTIC EXCELLENCE

Table of Contents

Intended Use

The Revolution cfDNA Max 20 Kit, Revolution cfDNA Max 20XL Kit, and Revolution cfDNA Reagent Kit are intended to extract cell-free DNA (cfDNA) from human plasma and urine using a semi-automated workflow on the Eppendorf ep*Motion* 5073 instrument. Use of the cfDNA Kits together with the Eppendorf ep*Motion* 5073 instrument as described in this document constitutes the Revolution Semi-Automated Workflow.

The Revolution semi-automated workflow is not labeled for IVD use, despite the fact that the Revolution cfDNA isolation kits are labeled for IVD use. The Revolution Semi-Automated Workflow is intended for researchers performing clinical research, and Research Use Only (RUO) applications.

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

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

Summary and Explanation

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

The procedures are suitable for nucleic acid isolation from human cell-free plasma or urine. Samples can be either fresh or frozen, although it is recommended that samples that have been previously frozen and thawed are not frozen again. The procedures are designed for minimal user handling, which enables the users to safely handle potentially infectious samples.

The isolated cfDNA is ready for use in downstream research applications, including PCR, real-time PCR (RT-PCR), and Next-Generation Sequencing (NGS). Alternatively, the purified cfDNA can be stored at -25°C to -15°C for later use.

Principles of the Procedure

Each Revolution cfDNA kit procedure includes the following steps:

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

Protease Treatment

Enzymes and other proteins are digested in the Revolution nRicher Cartridge.

Bead Binding

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

Bead Capture

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

Bead Washing

The Microvial Tube is removed from the Revolution nRicher Cartridge and placed into the Revolution Microvial Tube Rack. The Tube Rack is then loaded onto the ep*Motion* 5073 instrument for automated wash and elution.

Elution

The cfDNA is eluted from the beads with Revolution cfDNA Elution Buffer by the ep*Motion* instrument automatically and is ready for downstream applications.

Materials Provided

Revolution cfDNA Max 20 Kit, 100131

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

Revolution cfDNA Max 20XL Kit, 100155

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

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

‡ Store at -25°C to -15°C upon receipt

Materials Provided (continued)

Revolution cfDNA Reagent Kit, 100119

- Revolution cfDNA Surfactant, 7mL, 10007
- Revolution cfDNA Lysis Buffer, 22mL, 10011†
- Revolution cfDNA Protease Powder, 40mg, 10012‡
- Revolution cfDNA Protease Buffer, 2mL, 10056
- Revolution cfDNA Binding Buffer, 160mL, 10018†
- Revolution cfDNA Magnetic Beads, 1.4mL, 10028
- Revolution cfDNA Wash Solution, 54mL, 10084
- Revolution cfDNA Elution Buffer, 3.5mL, 10030
- Instructions for Use (online)

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

‡ Store at -25°C to -15°C upon receipt

Materials Required but Not Provided

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

Revolution equipment

- Revolution Processor, 10081
- Revolution Cartridge Rack, 100305
- Revolution Mag Capsules, 10080
- Revolution Mag Rack, 10082
- Revolution Microvial Tube Rack, 100462

Additional equipment, scripts, materials and reagents

- Eppendorf ep*Motion* 5073 instrument
- Revolution Sample Prep Semi-Automated Workflow scripts for the Eppendorf epBlue™ Studio software included with the ep*Motion* 5073 instrument
- Pipettors†, pipet tips‡ and serological pipettes
- Centrifuge† and Microcentrifuge†
- Non-magnetic microvial rack
- Vortex instrument with 2mL microvial tube adaptor
- 80% ethanol

† 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 combines simplicity with foundational technology to deliver unprecedented cfDNA extraction. See the Microvial Tube attachment to Sample Tube and cap.

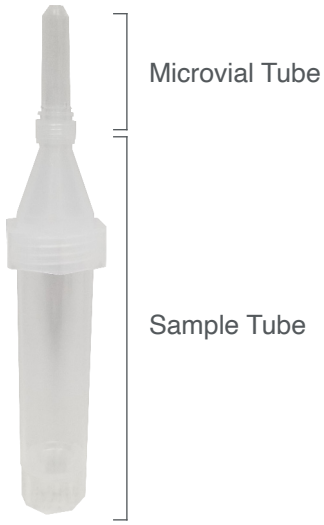


Figure 1
nRicher Cartridge

Figure 2
Removing Microvial Tube

Figure 3
Mag Capsule positioned on nRicher Cartridge

Figure 4
nRicher Cartridges 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

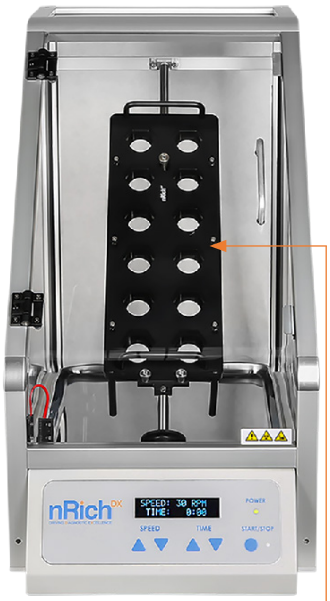


Figure 5
The Cartridge Rack is inserted into the Processor

Figure 6
The Revolution Microvial Tube Rack holds up to 24 nRicher Cartridge Microvial Tubes and is used with the Eppendorf ep*Motion* instrument

Reagent Storage and Handling

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

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

Sample and Reagent Volumes

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

- Green square (■) for **5 mL** sample volume
- Blue circle (●) for **10 mL** sample volume
- Tan diamond (◆) for **15 mL** sample volume
- Red triangle (▲) for **20 mL** sample volume

For example, ■ 250 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 cfDNA 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 cfDNA Surfactant, incubate the Surfactant at 37°C until the precipitate dissolves. This can happen if storage temperatures are too low.
- Vortex the Revolution cfDNA Magnetic Beads to fully resuspend them immediately before use.
- Blood samples should be collected in K2EDTA tubes or Streck Cell-Free DNA BCT tubes.
- Cell-free DNA is stable for 24 hours in K2EDTA plasma tubes stored at 2°C to 10°C.
- Blood samples collected in Streck Cell-Free DNA BCT tubes remain stable for up to 14 days due to the formaldehyde-free preservative in the tubes.
- Use fresh urine stored at 2°C to 10°C and spun down to cell-free status within 24 hours of collection to avoid increases in genomic DNA (gDNA) and microbial growth.
- Cell-free urine can be stored at 2°C to 10°C for up to 3 days without degradation.
- If it is not possible to process urine samples immediately after collection, cfDNA urine perservative should be added. Samples collected in the preservative should preferentially be stored and centrifuged per manufacturer recommendations.

Procedure

1. Protease 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. Sample Preparation

For Plasma Samples

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

2.2. Transfer the plasma to a new centrifuge tube.

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

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

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

2.5. All samples must be equilibrated to one of the starting sample volumes (e.g., 5, 10, 15, 20 mL) based on the following criteria:

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

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

For Urine Samples

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

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

2.9. All samples must be equilibrated to one of the starting sample volumes (e.g., 5, 10, 15, 20 mL) based on the following criteria:

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

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

3. nRicher Cartridge Preparation

3.1. Label the nRicher Cartridge elements (both Sample Tube and Microvial Tube; see Figure 1, page 8) with a sample identifier; use one nRicher Cartridge per sample.

3.2. Place the nRicher Cartridge(s) into the Cartridge Rack.

NOTE: The cartridges should be left in the rack for the entirety of the extraction.

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

4. Protease Treatment

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

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

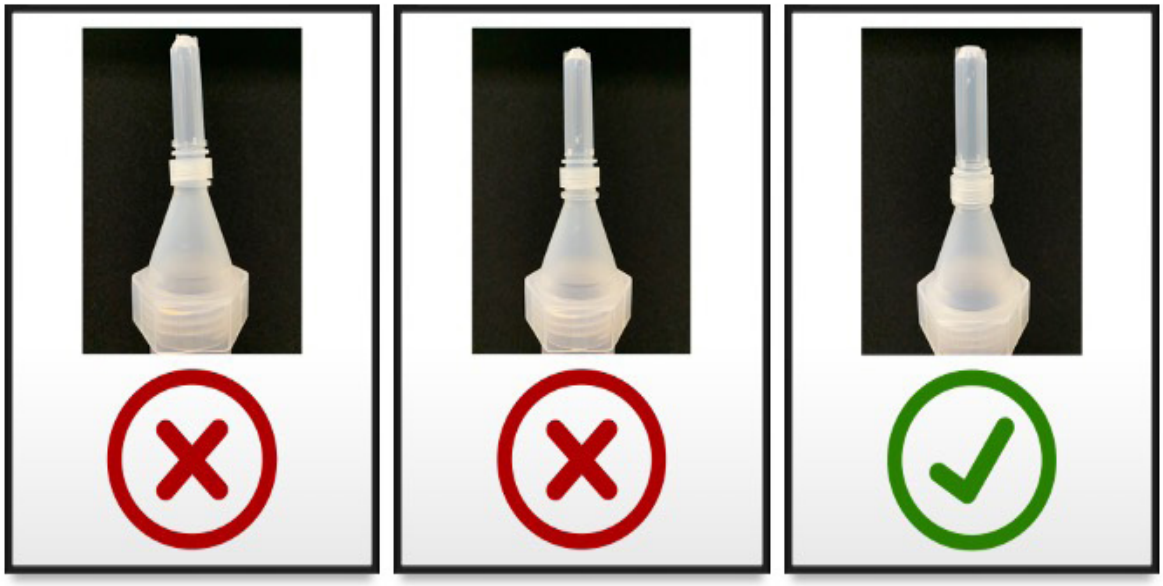
4.3. Add ■ 800 µL, ● 1600 µL, ◆ 2400 µL, or ▲ 3200 µL Lysis Buffer to each Sample Tube.

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

4.5. Close the nRicher Cartridge(s) by attaching the Microvial Tube.

CAUTION: Do not over tighten.

NOTE: A tip for connecting the two parts. 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.



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

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

4.8. When the Processor stops, remove the Cartridge Rack from the device, place the rack on a level surface, and incubate for another 20 minutes at room temperature.

5. Bead Binding

5.1. Remove the Microvial Tube from each nRicher Cartridge and place it into a separate non-magnetic microvial rack.

5.2. Add ■ 6 mL, ● 12 mL, ◆ 18 mL, or ▲ 24 mL Binding Buffer to each Sample Tube.

5.3. Resuspend the Revolution cfDNA Magnetic Beads by vortexing at medium speed for 10 seconds.

5.4. Add ■ 50 µL, ● 100 µL, ◆ 150 µL, or ▲ 150 µL Magnetic Beads to each Sample Tube.

5.5. Attach the Microvial Tube to its companion nRicher Cartridge, and then transfer the Cartridge Rack containing the nRicher Cartridges to the Revolution Processor.

5.6. Start the Processor and incubate at room temperature (15°C to 30°C) at 30 rpm for ■ 30 minutes, ● 45 minutes, ◆ 45 minutes, or ▲ 45 minutes.

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

6. Bead Capture

6.1. Snap a Mag Capsule onto each nRicher Capsule (see Figure 3, page 8), and then place the Cartridge Rack into the Revolution Processor.

6.2. Start the Processor and incubate at room temperature (15°C to 30°C) at 30 rpm for ■ 15 minutes, ● 20 minutes, ◆ 30 minutes, or ▲ 30 minutes.

6.3. When the Processor stops, remove the Cartridge Rack from the device, and place the rack on a level surface; let the rack sit for 1 minute to allow all of the liquid to drain from the Microvial Tube into the Sample Tube.

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

6.5. Orient the Mag Capsule and Microvial Tube so that the rounded bottom of the Microvial Tube is facing downward; then press the Mag Capsule down toward the benchtop to release the microvial tube.

6.6. Transfer the Microvial Tubes to a microcentrifuge and spin for approximately 2 seconds. This will collect the bead solution to the bottom and make it much easier to resuspend in the ep*Motion* system. **NOTE: Discard the liquid remaining in each Sample Tube along with the Sample Tube itself in biohazardous waste.**

6.7. Transfer the tubes to the nRich microvial tube rack, then place the entire rack in the C2 position of the ep*Motion* 5073 instrument.

NOTE: Microvial tubes should be placed in order as shown below:

7. Automated Bead Washing and Elution on the ep*Motion*

7.1. Prepare the ep*Motion* system with items in the following positions on the deck of the ep*Motion* instrument *(see also diagrams below)*:

7.1.1. A1: TMX position/Mixing platform with an Eppendorf 2 mL deep-well-plate on top

7.1.2. A2: p1000 Filtered tip box

7.1.3. B1: p1000 Filtered tip box *(Do not place a tip box here if running 8 or less samples)*

7.1.4. B2: Reagent rack with p50 or p300 tips in columns 4, 5, and 6

- Column 1: 100 mL reservoir with Wash Buffer
- Column 2: 100 mL reservoir with 80% EtOH
- Column 3: 30 mL reservoir with Elution Buffer

- Columns 4-6: p50 Eppendorf tips (use p300 tips for 100 µL elution volume)

7.1.5. C1: Eppendorf Magnum FLX magnet

7.1.6. C2: nRich microvial tube rack with tubes

7.1.7. G1: Gripper tower with gripper

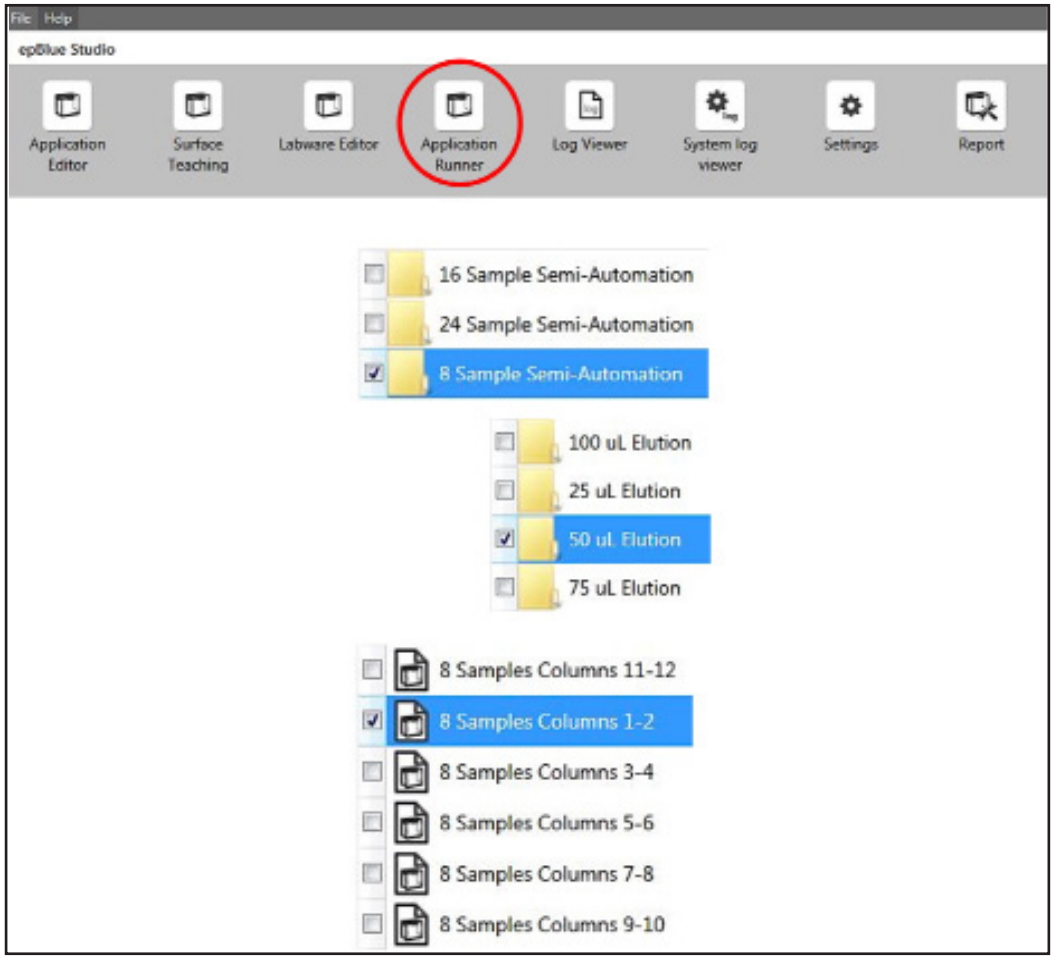
7.1.8. T1, T2, & T3: These are all interchangeable. Make sure to have multi-channel p50 (or p300 for 100 µL elution) and p1000 pipettes as well as a single channel p1000 pipette.

7.1.9. Waste: Make sure the waste bin is positioned in the appropriate area.

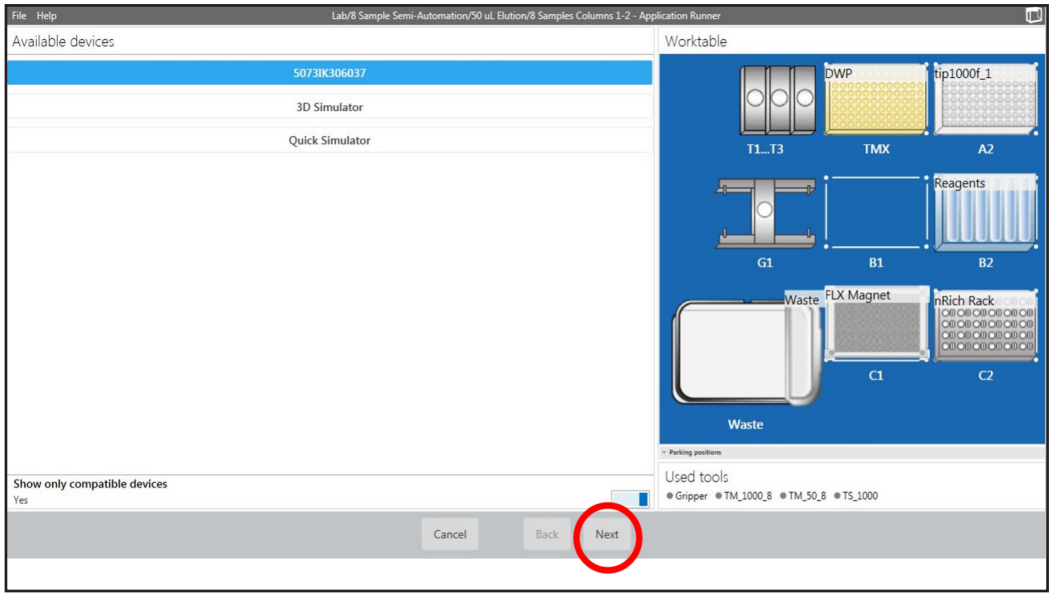
NOTE: Make sure the waste bin is completely empty before starting the automation.

NOTE: If user is running more than 8 samples, it is recommended to have two full p1000 tip boxes. ALWAYS make sure that p50/p300 tips are completely filled.

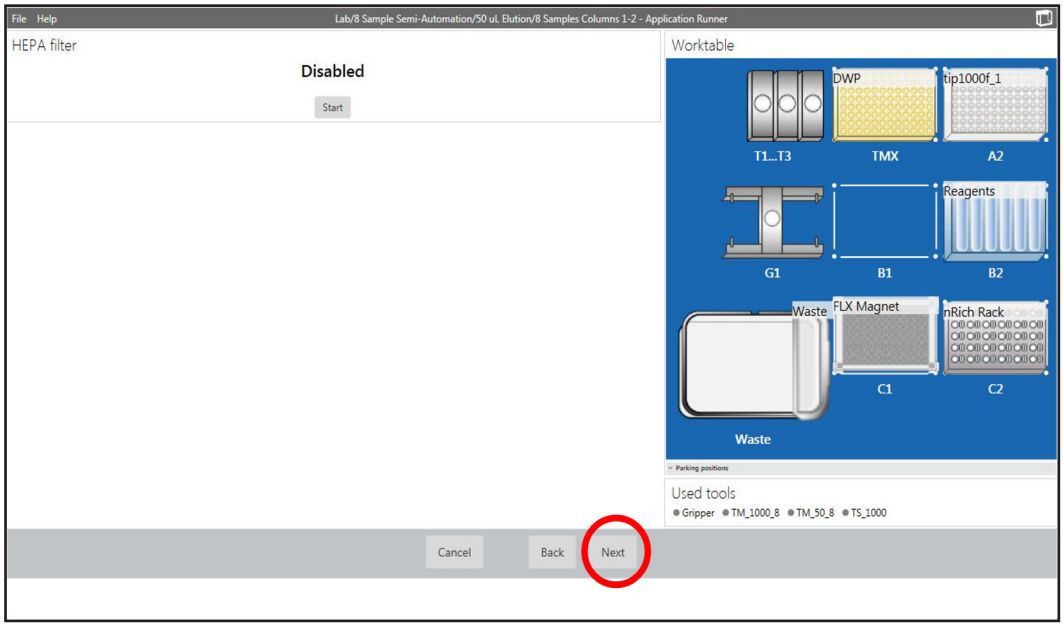
7.2. Open the epBlue Studio application and select the application runner from the home screen. User will then be prompted to select the desired script based on their number of samples, desired elution volume, and available columns on their deep-well-plate. These are all shown on the next page with the example selecting: application runner > 8 sample semi-automation > 50 µL elution volume > columns 1-2:



7.3. After double-clicking the script, the system will ask to run the program. Select the top option which should be the identification number for the user's ep*Motion* 5073 system. Click "Next".



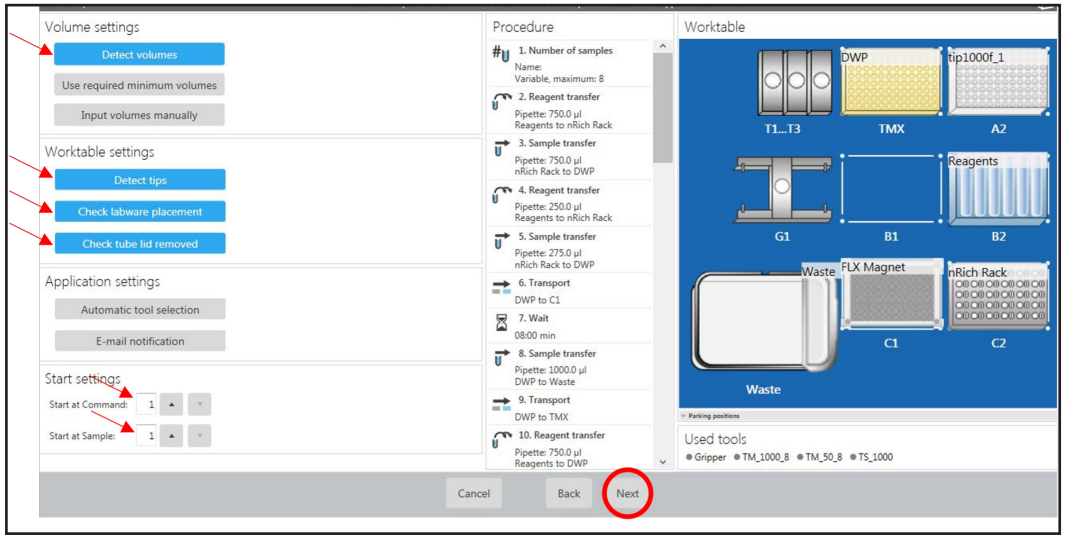
7.4. The software will ask to enable or disable the HEPA filter. Select the user's desired command and click "Next".



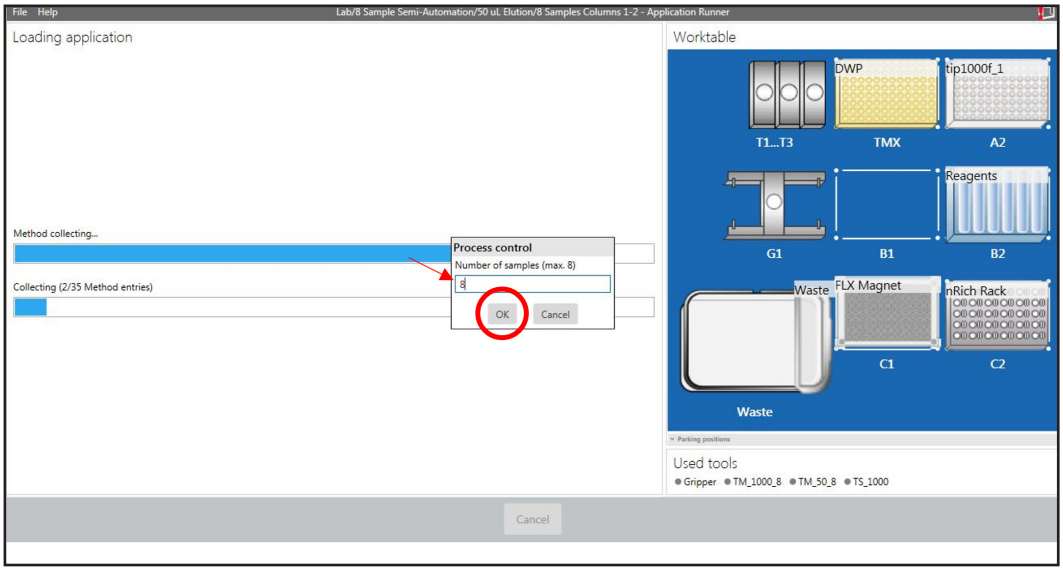
7.5. It will ask which values should be detected and upon which step to start. Ensure the following are clicked and appear blue:

- Detect Volumes
- Detect Tips
- Detect Labware Placement
- Detect Tube Lids

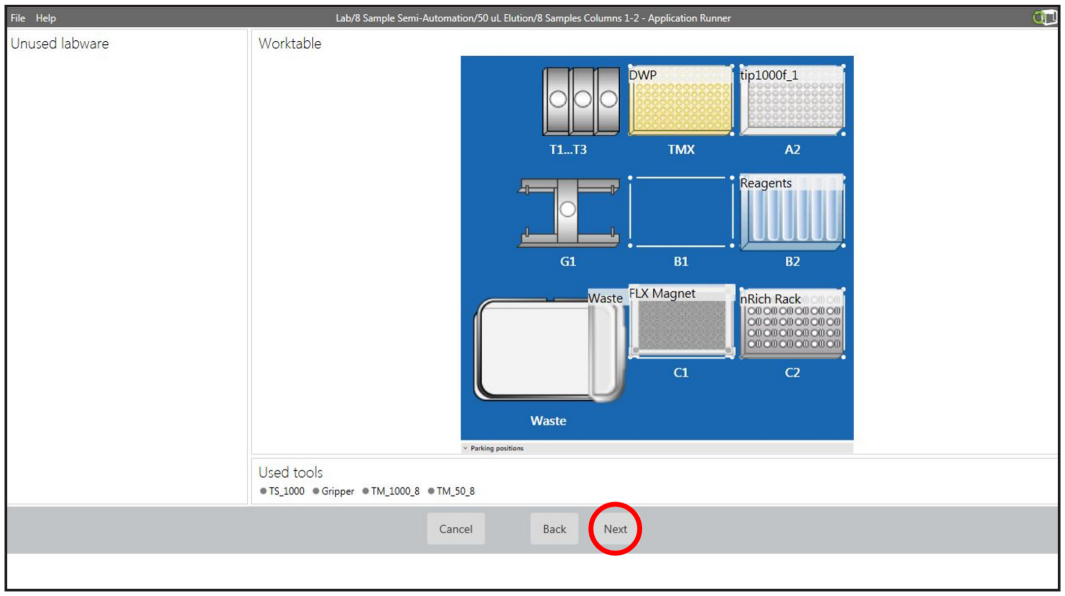
Also, ensure that the start position for both sample and step are both at 1.



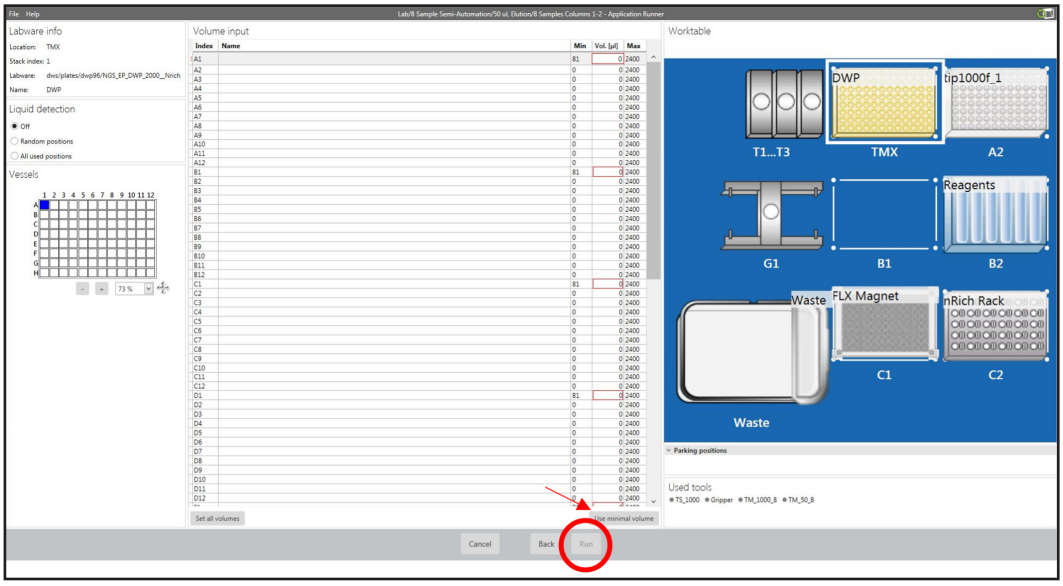
7.6. The epBlue software will require number of samples to be run. Input your number of samples and click “OK” (“8” samples are shown in the example below).



7.7. Next, verify the ep*Motion* worktable. If the virtual representation appears as specified in Step 7.1, click “Next”.



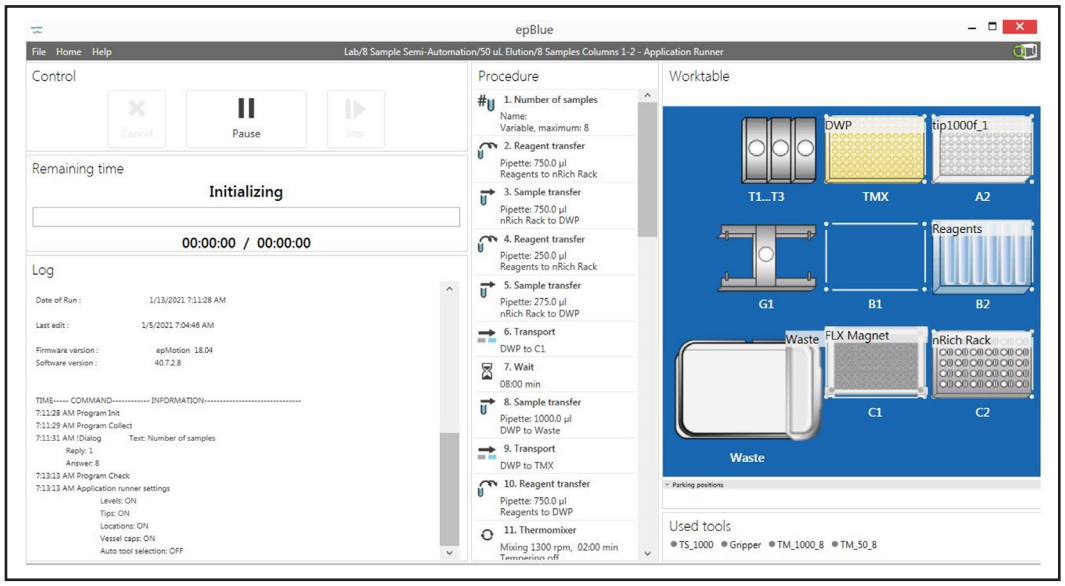
7.8. The software will ask about the volume currently in the deep-well-plate. Click on the button at the bottom right of the screen, “Use minimal volume” and click “Run”.



7.9. At this point the ep*Motion* 5073 will go through a short initializing process. Once it has successfully initialized, it will provide an estimated time to completion on the screen. NOTE: Time to completion is an estimate. As the automation continues the time will update.

Average sample times after initialization:

- 8 Samples: 1 hour, 36 minutes
- 16 Samples: 2 hours, 12 minutes
- 24 Samples: 2 hours, 47 minutes



7.10. After the run has completed, a message will appear on the screen. Press “Ok” and remove the deep-well-plate from position C1. The eluted sample should be in the adjacent well to the beads (e.g. if the script for columns 1-4 was chosen by the user, beads will be in columns 1 & 3 while the eluant will be in columns 2 & 4).

The Revolution Sample Prep Semi-Automated Workflow is complete. Transfer the eluate into a clean microvial PCR tube.

7.11. The eluant is now ready for downstream research applications. Store the eluate at -20°C until ready for testing.

7.12. Discard the microvials containing beads as biohazardous waste.

Troubleshooting

Observation	Possible cause	Recommended action
Lower yield than expected	The Revolution cfDNA Magnetic Beads were not properly stored	Store the Revolution cfDNA Magnetic Beads at 15°C to 30°C. Do not freeze the beads.
	An insufficient amount of Revolution cfDNA Magnetic Beads was added	Immediately before use, vortex the tube containing the magnetic beads thoroughly until fully resuspended.
	The Revolution cfDNA Magnetic Beads are not optimally dried	Drying times may vary depending on the amount of beads used and the environment. Lower volumes of beads require less time to dry. Airflow and humidity in the immediate environment may shorten or lengthen the optimal bead drying time.
		Overdried beads will stick to the wall of the tube and be difficult to re-suspend. Gently scrape the beads off the plastic wall using a pipette tip.
		Underdried beads may carry ethanol into the eluate and negatively impact downstream applications. Dry beads slightly longer (1-minute intervals) and make note of the optimal drying time for the specific volume.
	The sample contains low levels of cfDNA	Increase the starting sample volume.
Magnetic bead carryover	Loose beads present in the eluate or inadvertently transferred	Be sure to leave the Microvial Tube(s) on the Mag Rack when removing the eluate containing the cfDNA.
		If beads are carried over into the new tube, place the tube on the Mag Rack again, wait for the beads to pellet and then transfer the sample to another tube.

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 12). Also see the Microvial Tube attachment attachment to Sample Tube and cap for reference.
Variations in cfDNA yield from donor to donor	Variation in amount of circulating cfDNA. Levels of cfDNA in circulation can range from 1 to 100 ng/mL of plasma or serum depending on the donor.	For samples containing low levels of cfDNA, increase the starting sample volume.

Technical Support

For additional questions, please contact technical support services at **support@nrichdx.com**.

Warnings and Precautions

For In Vitro Diagnostic Use

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

Discard all used materials as biohazardous waste according to local regulations. CAUTION: The Revolution Lysis Buffer contains guanidinium thiocyanate, which when combined with bleach, forms highly reactive compounds.

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

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

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

Revolution cfDNA Surfactant

Hazard pictograms: 

Signal word: DANGER

Hazard and precautionary statements:

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

Revolution cfDNA Lysis Buffer

Hazard pictograms: 

Signal word: DANGER

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

Revolution cfDNA Protease

Hazard pictograms: 

Signal word: DANGER

Hazard and precautionary statements:
H315: Causes skin irritation; H319: Causes serious eye irritation; H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled; H335: May cause respiratory irritation; P261: Avoid breathing dust / fumes / gas / mist / vapors / spray; P305 + P351 + P338: IF IN EYES - Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing; P341 + P311: If experiencing respiratory symptoms: Call a POISON CENTER or doctor / physician.

Revolution cfDNA Protease Buffer

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 1. Sample and Reagent Volumes

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

Table 2. Bead Binding and Bead Capture Incubation Times

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

Symbols

Research Use Only. Not for use in diagnsotic procedures

Catalog numbers

Manufacturer

Use-by date

Batch code

Consult instructions for use

Caution