liquidbio

# Instructions for Use

# IsoFlux NGS DNA Kit

## Applies to: 910-0104

**Updated:** 2023-04-01
**Revision:** D

## INTENDED USE

The IsoFlux NGS DNA Kit is intended to be used with the IsoFlux System, a benchtop instrument for semi-automated cell isolations, and one of the IsoFlux enrichment kits. Following enrichment of circulating tumor cells (CTCs) or other rare cells from blood samples, the NGS DNA Kit further enhances the purity of target cells and performs whole genome amplification (WGA), hence generating DNA samples amenable to NextGeneration Sequencing (NGS). The IsoFlux System and associated products are intended for research applications only.

## SUMMARY

The IsoFlux NGS DNA Kit workflow has two components: purity enhancement and whole genome amplification (WGA). The purity enhancement column and reagents deplete contaminating leukocytes from the magnetic bead pellet following a standard IsoFlux enrichment procedure, enabling high target cell purity that is required by NGS analysis. The amplified gDNA, at a typical concentration of approximately 50ng/µL, should be column cleaned-up to remove residual salts and proteins, or diluted to at least 1:50 before using for NGS. We recommend using QIAamp DNA Micro Kit (Qiagen Catalog no. 56304), and using the supplementary protocol (downloadable from Qiagen's website, or see below) to clean up the WGA product prior to use.

https://www.qiagen.com/us/resources/faq?id=fcc46a72-d1f4-4cec
b6bbfe0ea9da5bae&lang=en

## KIT CONTENTS AND STORAGE INFORMATION

| Box | Component | Amount | Shipping Condition | Storage & Stability |
|---|---|---|---|---|
| Purity Enhancement Columns | Column | 24 pieces | Room Temperature | Room Temperature |
| | Cap | 24 pieces | | |
| | Stopper | 48 pieces | | |
| | Pedestal | 24 pieces | | |
| | 50mL Tube | 48 pieces | | |
| | 1.5mL Tube | 24 pieces | | |
| Purity Enhancement Reagents | Blocking Buffer (10x) | 25mL | Ice | 4-8°C. Stable for at least one year. |
| | Release Buffer | 11mL | | 4-8°C, protected from light. Stable for at least one year if kept sterile. |
| | DNA Polymerase (blue lid) | 100µL | Dry Ice | Store upon receipt at -80°C (stable for at least one year). Store at -20°C after opening (stable for at least 6 months). |
| | Reaction Buffer (yellow lid) | 2 x 1.45mL | | |
| | KOH Buffer (clear lid) | 2 tubes | | |
| | Stop Solution (red lid) | 1.8mL | | |
| | PBS, 1x (clear lid) | 1.8mL | | |
| | DTT, 1M (lilac lid) | 1mL | | |

## MATERIALS REQUIRED, NOT PROVIDED

- IsoFlux Instrument (P/N 950-0100)
- One of the IsoFlux enrichment kits (P/N 910-0091, 910-0092, 910-0106, or 9100108) Dulbecco's Phosphate-Buffered Saline (DPBS) without Ca2+ and Mg2+ PCR tubes
- Tube racks
- Tube rotator
- Centrifuge
- Serological pipettes and pipettor
- Calibrated micro-pipettes and tips
- Micro-centrifuge tubes
- Nuclease-free water
- Thermocycler
- Clean, lint-free tissue (KimWipe; KimTech Catalog no. S-8115)

### WARNINGS AND PRECAUTIONS

- For Research Use Only.
- Please read the entire contents of these Instructions for Use before processing samples.
- All personnel should follow universal precautions for biological sample handling and use laboratory safety equipment (i.e., safety goggles, laboratory coat, and disposable gloves).
- All materials coming in contact with the specimen(s) are considered biohazardous. Handle as if capable of transmitting infection. Treat and dispose of waste using proper precautions and in accordance with local, state, and federal regulations. Never pipette by mouth.
- KOH Buffer contains potassium hydroxide: Corrosive. Harmful if swallowed. Can cause severe burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

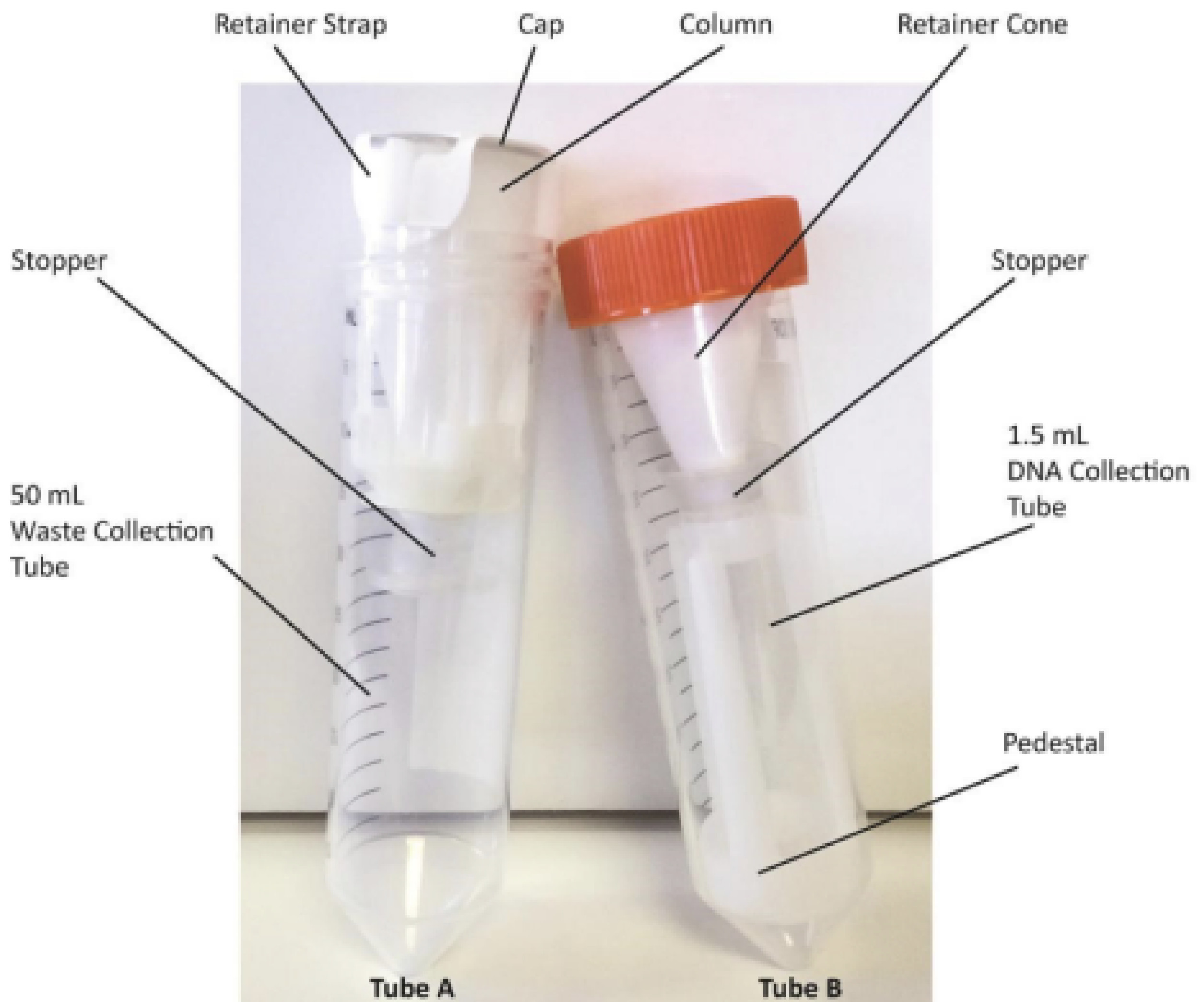


**Figure 1.** Purity Enhancement Column setup: Each sample requires one Tube A and one Tube B. Tube A houses the column assembled with its cap and stopper. The 50 mL tube itself also served as a waste collection vessel. After the purity enhancement workflow is completed, the column will be transferred to Tube B for the WGA workflow. Tube B (orange cap) houses an extra clean stopper, a pedestal, and the 1.5 mL DNA collection tube.

## EXPERIMENTAL PROCEDURE



### Blocking

1. The purity enhancement columns (Figure 1) are packaged within Tube A (50 mL waste collection tube). They are designed to stay within this tube during the purity enhancement workflow. Before an experiment, first remove the retainer strap by gently pulling the stopper off.
2. For each sample, remove the cap from one column and re-insert its stopper. Label the column and place it on a tube rack.
3. Dilute 1 mL of 10x Blocking Buffer with 9mL DPBS. Add 9mL into the column reservoir.
4. Insert the cap securely and incubate at room temperature for ≥ 30 minutes. Set aside until use. *In the meantime go to Step 1 of Purity Enhancement procedure below.*

5. Lift the column, invert, remove the stopper, and revert back into the 50mL tube. Remove the cap.
6. Allow the Blocking Buffer to drain completely and then discard the waste. 7. The column is now ready to receive samples.

**Purity Enhancement**

1. Remove the Release Buffer tubes from the Purity Enhancement Reagents box (48°C). Each tube corresponds to one sample. Label the tubes with appropriate sample information, and warm the Release Buffer to 37°C. The Release Buffer is overfilled to 11mL. Reduce the volume to 10mL prior to proceeding to next step.
2. After the IsoFlux enrichment procedure, pipette ~30µL Binding Buffer into the Low Volume Recovery Holder and completely transfer the beads/pellet into the pre-warmed tube of Release Buffer. Rinse the Low Volume Recovery Holder at least 3 times with Release Buffer to collect any residual beads.
3. Screw on the cap tightly and incubate at room temperature on a rotator for 30 minutes.
4. Proceed to prepare the reagents as described in step 1 to 3 of the Whole Genome Amplification (WGA) protocol below.
5. After incubation, slowly pour Release Buffer containing the sample into the blocked column, making sure that sample does not overflow. Re-cap the Release Buffer tube and set aside on a tube rack.
6. Allow the sample to completely drain by gravity flow.
7. Add 10mL DPBS into the Release Buffer tube. Screw on the lid to close. Invert several times to rinse the tube. Slowly pour the rinse into the sample column. Use a pipette to transfer any remaining liquid into the column with to ensure complete sample extraction.
8. After the liquid flows through completely, rinse the column once more with 10mL DPBS. Allow the rinse to completely drain by gravity flow.
9. Gently wick away excess DPBS at the bottom of the column using a clean, lint free Kimwipe. ***It is important to remove all residual liquid and air bubbles under the membrane.***
10. Open Tube B. Remove the retainer cone and insert the clean stopper into the bottom of the column. It is important to ensure a snug fit. Proceed immediately with Step 4 of the Whole Genome Amplification protocol as described below.

**Whole Genome Amplification**

1. Set a water bath, heat block, or thermocycler at 30°C
2. Thaw the DNA Polymerase on ice. Thaw all other reagents at room temperature. Vortex the tubes and centrifuge briefly. The Reaction Buffer and DTT solution may form a precipitate, which will dissolve after vortexing for 10 seconds. Place all reagents on ice after thawing.
3. If using the kit for the first time, reconstitute the KOH Buffer by adding 500µL nuclease free water to the tube. Mix thoroughly and centrifuge briefly. *The KOH Buffer is pH*

*labile. Avoid neutralization with CO2.* Stored the reconstituted KOH buffer at -20°C in aliquots. It is stable for 6 months.

4. *The following preparation is per 4 samples. We recommend preparing for at least 4 samples. Scale up as appropriate. Avoid scaling down. Lysis buffer may be stored at 20°C* ***and used within 3 months.*** Prepare the following reagents and keep on ice until use.

**Preparation for Lysis Buffer:**

**Component Volume**

DTT 5µL

KOH Buffer (reconstituted) 55µL

Total volume 60µL

1. For each sample add **14µL of Lysis buffer** onto the center of the circular membrane by lowering a micropipette into the column reservoir until its tip hovers directly above the membrane. Use visual guidance as necessary.
2. Bring the volume to total 26µL by adding up to **12µL of PBS or nuclease-free water** onto the center of the membrane. Pipette up and down 5 times and avoid making bubbles, aspirating from the periphery of the membrane and dispensing into the center. *This is important to facilitate even spreading of the Lysis Buffer across membrane and complete lysis of cells.* Avoid puncturing the membrane with the pipette tip.
3. Incubate the column on ice or at 4°C for 10 minutes.
4. Add **14µL** of Stop Solution. Gently pipet up and down twice.

**Preparation of WGA reaction PER SAMPLE** ***(Use the following table as a guide)***



**Component Volume per sample** Cell lysate (in column from step 8 above)

40µL

Reaction buffer 116µL

Nuclease-free water 40µL

DNA Polymerase* (add only prior to incubation at 30°C ) 4µL

Total volume 200µL

1. Add **116µL of Reaction Buffer**. Add up to **40µL of nuclease-free water** to bring the total reaction volume to 196µL. Pipette up and down 5 times.
2. Transfer by pipetting as much as possible of the reaction mix into a new clean PCR tube (0.2mL, 0.5mL, or 1.5mL microfuge tubes may be used; not provided). Close the lid and set aside on ice.
3. Remove the stopper from the column and secure the DNA collection tube below the column. Place this assembly over the pedestal in Tube B.
4. Centrifuge at 1000xg for 1 min.
5. Disassemble the apparatus and transfer the remaining reaction mix into the same PCR tube in step 10. Label the tube accordingly.
6. Add 4µL of DNA Polymerase per sample. Mix by very gently pipetting up and down several times or brief vortex and centrifugation. Incubate at 30°C for 10 to 16 hours.
7. After incubation at 30°C, heat the water bath or heating block up to 65°C if the same water bath or heating block were used in step 14.*(Note: If a thermal cycler is used with a heated lid, temperature of the lid should be set to 70°C.)* Inactivate DNA Polymerase by incubating at 65°C for 3 minutes.
8. The amplified gDNA can now be stored at -80°C, and may be used directly in NGS without the need of purification. Minimize freeze-thaw cycles. However, we recommend column cleaned-up to remove residual salts and proteins, or diluted to at least 1:50 before using for NGS. We recommend using QIAamp DNA Micro Kit (Qiagen Catalog no. 56304) to clean up the WGA product.
9. Concentration determination of amplified gDNA requires quantification methods specific for double-stranded DNA, since the amplification products contain unused reaction primers. Typical concentration is approximately 50ng/µL.
10. For PCR analysis, dilute amplified gDNA 1:25 with nuclease free water, and load 2 to 3µL in each PCR reaction.
11. We recommend using TaqMan™ RNase P Detection Reagents Kit (Thermofisher 4316831) and Premix Ex Taq (Probe qPCR) Master Mix (Takara RR39LR) or equivalent to assess the WGA product prior to performing library prep for NGS.