

### IsoFlux™ NGS DNA Kit

High purity sample preparation kit enables NGS from IsoFlux-enriched blood samples

#### OVERVIEW

- Seamless integration with IsoFlux System workflow
- Enhances purity through leukocyte depletion process
- Amplifies DNA using highly uniform and accurate process
- Produces an abundance of gDNA ( $\mu\text{g}$  quantities) for use in NGS
- Enables stable, long-term storage of gDNA



#### BACKGROUND

Enrichment of tumor material from the peripheral blood circulation holds tremendous potential for cancer biomarker discovery and development. The primary limitation thus far has been the purity and number of target copies in the samples. Next-generation sequencing (NGS) and other genomic analyses typically require 5% or greater purity to reliably detect the target <sup>1</sup>. Many of the approaches to capturing tumor material from the circulation deliver purity below 1%, and often below 0.1% <sup>2</sup>.

The IsoFlux NGS DNA Kit addresses this limitation by further increasing the purity of tumor cell samples enriched with the IsoFlux System. The kit also incorporates a Whole Genome Amplification (WGA) step to produce abundant quantities (1-10 $\mu\text{g}$ ) of gDNA using an accurate and uniform DNA polymerase. This gDNA material is suitable for long-term storage and analysis using NGS and comparable genomic methods.

#### PRINCIPLES OF OPERATION

The IsoFlux System enriches circulating tumor cells from peripheral blood samples using immunomagnetic beads targeted towards cell surface receptors specific to tumor cells. The enriched tumor cells are captured in a microfluidic cartridge and presented to the user as a low-volume (3 $\mu\text{L}$ ) cell droplet. This droplet is easily accessed using a pipette and is transferred directly into the IsoFlux NGS Kit workflow.

The IsoFlux tumor cell droplet is placed into the Purity Enhancement Column. The retained target cells are washed and recovered using the supplied buffers, followed by cell lysis.

The cell lysate then goes through the WGA process. This uses the REPLI-g<sup>®</sup> DNA polymerase that ensures highly uniform and accurate whole genome amplification from small samples in just 60–90 minutes. The WGA process results in typical DNA yields of 1-10 $\mu\text{g}$  per reaction.

#### ORDERING INFORMATION - WHAT'S IN THE BOX

##### IsoFlux NGS DNA Kit (Cat# 910-0104) - 24 samples

- Purity Enhancement Columns (Qty. 24)
- Running buffer
- REPLI-g<sup>®</sup> DNA Polymerase, Buffers, and Reagents (for 24 x 80 $\mu\text{l}$  ultrafast whole genome amplification reactions)



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#### samples PROCEDURE

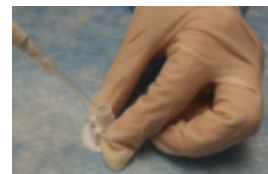
##### 1. Retrieve tumor cell pellet from IsoFlux microfluidic cartridge

Transfer cell pellet into Purity Enhancement Column loaded with Release Buffer. Insert cap and incubate at room temperature for 30 minutes with rotation.



##### 2. Run cells through Purity Enhancement Column

Remove stopper and cap from Purity Enhancement Column. Allow sample to drain into collection tube by gravity flow.



##### 3. Wash with supplied buffer

Wash column twice with PBS. Centrifuge at 50x g for 1 minute to remove residual buffer. Insert clean stopper.



##### 4. Add Lysis Buffer to Purity Enhancement Column

Incubate at 4°C for 10 minutes. Add Stop Solution, mix well. Add WGA master mix, mix well.

##### 5. Aspirate sample from the column using a micro-pipette

Dispense into the supplied microfuge tube. Remove the stopper, secure the microfuge tube below the column, and centrifuge briefly to collect residual sample.



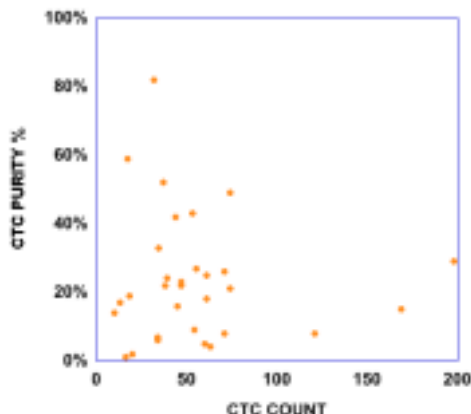
##### 6. Incubate at 30°C for 4 hours

Heat the sample for 3 min at 65°C to inactivate the enzyme.

##### 7. Collect WGA product (~120µL) and store at -20°C.

Aliquot gDNA as needed for NGS and other genomic analysis.

#### ANALYTICAL VALIDATION

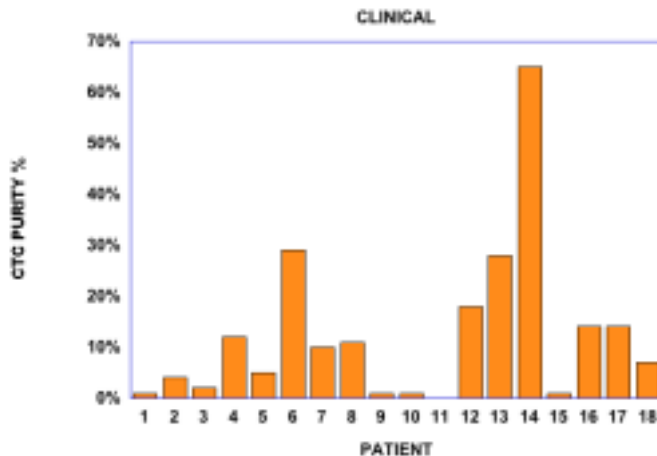


**ANALYTICAL VALIDATION:** A series of 30 healthy blood samples (multiple donors) were spiked with decreasing levels of a tumor cell line (MDA-MB-231). The overall tumor cell purity was >5% in 90% of the samples.

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#### CLINICAL VALIDATION



**CLINICAL VALIDATION:** A group of 20 CTC-positive (CTC>10) clinical samples from multiple indications (prostate, bladder, colorectal) were processed with the IsoFlux NGS DNA Kit and enumerated. 80% of the samples (16/20) had higher than 5% purity, above the detection limit of most NGS assays.

#### REFERENCES

1. Tsongalis, et al. "Routine use of the Ion Torrent AmpliSeq™ Cancer Hotspot Panel for identification of clinically actionable somatic mutations". Clin Chem Lab Med 2014; 52(5): 707–714
2. Zhe, et al. "Circulating tumor cells: finding the needle in the haystack". Am J Cancer Res. 2011; 1(6): 740–751.

#### ORDERING INFORMATION

Contact [orders@liquidbio.co](mailto:orders@liquidbio.co) for ordering information.

#### MORE INFORMATION

[www.liquidbio.co](http://www.liquidbio.co)

[info@liquidbio.co](mailto:info@liquidbio.co)

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