AccuGenomics
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PRODUCT INSERT

Accukit™ ctDNA MRD IS

SNAQ™-SEQ Spike-in Standards for MRD

PRODUCT AND INTENDED USE:

The Accukit™ ctDNA MRD IS product is a reference material co-developed with LGC Diagnostics and Genomics Innovation Hub that is spiked into each sample in Next Generation Sequencing (NGS) assays that detect somatic mutations in human cancer patient samples. This product is intended for use as a quality control material in the development, validation and routine testing of laboratory tests used to monitor cancer disease progression or treatment response by ctDNA NGS assays. Product is manufactured for Research Use Only (RUO). Not for use in diagnostic procedures.

REAGENTS:

Catalogue Number:	Product Name:
2091	Accukit™ ctDNA MRD IS

Accukit™ ctDNA MRD IS consists of 1 x 10µL vial of reagent at a concentration of 300 copies/µL for each target.

WARNINGS AND PRECAUTIONS: For Research Use Only (RUO). Not for use in diagnostic procedures.

Caution: While Accukit™ ctDNA MRD IS consists of synthetic DNA constructs, you should handle it as though it can transmit infectious agents.

SAFETY PRECAUTIONS: Use Centers for Disease Control and Prevention (CDC) recommended universal precautions for handling reference materials and human specimens[1]. Do not pipette by mouth. Do not smoke, eat, or drink in areas where specimens are being handled. Clean any spillage by immediately wiping with 0.5% sodium hypochlorite solution. Dispose of all specimens and materials used in testing as though they contain infectious agents.

HANDLING PRECAUTIONS: Do not use the Accukit™ ctDNA MRD IS reagents beyond the expiration date. Avoid contamination when opening or closing the vial. Use low retention tubes and pipette tips and nominal pipetting volumes when working with Accukit prior to sample addition.

STORAGE INSTRUCTIONS: Store Accukit™ ctDNA MRD IS frozen at -20 C. User may aliquot the product (use low DNA binding tips and tubes), especially if the planned product use will require > 5 freeze-thaw cycles.

INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION: Accukit™ ctDNA MRD IS consists of synthetic DNA and should appear as a clear liquid. Any change in this appearance may indicate instability or deterioration of the product and vials should be discarded.

MATERIALS PROVIDED: The Accukit™ ctDNA MRD IS reagents consist 1 x 10uL vial of fragmented synthetic DNA (130 bp to 210 bp). The DNA is provided in 10 mM Tris, 0.1 mM EDTA, pH 8.0 buffer.

OTHER REQUIRED MATERIALS: Refer to instructions provided by the manufacturer of the test kit to be used.

INSTRUCTIONS FOR USE: Thaw the Accukit™ ctDNA MRD IS vial. Mix to ensure a homogeneous mixture and spin briefly to collect. The amount of material to spike into each sample is dependent on the limit of detection (LOD) of the method. One approach is to add sufficient low levels of Internal Standard (IS) while still ensuring consistent IS variant detection under normal testing procedures. If your test LOD was based on a variant failure rate with nominal sample concentrations and that variant is not associated with IS regions, then add sufficient IS to achieve a median IS Variant Allele Frequency (VAF) at the LOD of your test. The formula below provides suggested starting IS copies to be added to each sample to achieve the desired IS VAF, with the IS_{copies} added to each sample based on the X ng of genomic DNA input and the desired Allele Fraction ($AF_{desired}$, a number between 0.0-1.0). For example, using a nominal input of 20 ng DNA with a LOD of 0.5% (i.e., a 0.005 $AF_{desired}$), use 30 copies of IS mixture added to the purified DNA sample prior to library preparation. After estimating the IS VAF arising from 6-8 nominal samples, it may be necessary to make a final adjustment of the IS input level to achieve the desired median IS VAF. Bear in mind the impact of stochastic sampling on variant detection when setting the IS level. Once IS input copies is established, always use the same IS spike-in level for each sample tested. To ensure accurate delivery of IS reagent, use a nominal pipetting volume.

$$IS_{copies} = AF_{desired} * \frac{\left(\frac{X\ ng}{0.0033\ \frac{copies}{ng}}\right)}{(1 - AF_{desired})}$$

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EXPECTED RESULTS AND INTERPRETATION OF RESULTS:

Table 1 (bottom) lists the unique altered IS sequences that are near clinically relevant mutations. The Standardized Nucleic Acid Quantification (SNAQ™) limit controls present in the Accukit™ ctDNA MRD IS product are designed to provide a direct indication of an NGS test's variant detection sensitivity for the regions covered by the IS. By adding an expected level of copies of a known sequence to each purified nucleic acid sample prior to library preparation, the IS variant detection provides a direct indication of the assay ability for each sample. Further, because the IS and sample yields biochemically covary, the IS VAF provides a direct measurement of sample abundance. Sample abundance is critical to NGS sensitivity assumptions, especially when sample quantity/quality are highly variable, such as with FFPE or cfDNA.

$$Sample\ Templates = (\frac{1}{IS_{VAF}} - 1) * IS_{COPIES}$$

Figure 1 (below) shows a similar ctDNA read fragment size distribution for the IS and sample template in the same region. The IS fragments make up 5.5% of the fragments in this region, near the expected 6% IS VAF based on the formula presented above. Detection of somatic mutations may differ across different NGS panels, and concomitantly the VAFs determined by targeted NGS panels for the Accukit ctDNA MRD IS product may differ. Each laboratory must establish an expected VAF for the somatic variants in the Accukit ctDNA MRD IS product. When results for the product are outside of the established acceptance range, it may indicate unsatisfactory sample or test performance. Possible sources of error include sample interference, deterioration of test kit reagents, operator error, faulty performance of equipment, contamination of reagents, or changes in bioinformatics pipeline parameters. Additional support documents are available by contacting us at info@accugenomics.com.

Figure 1:

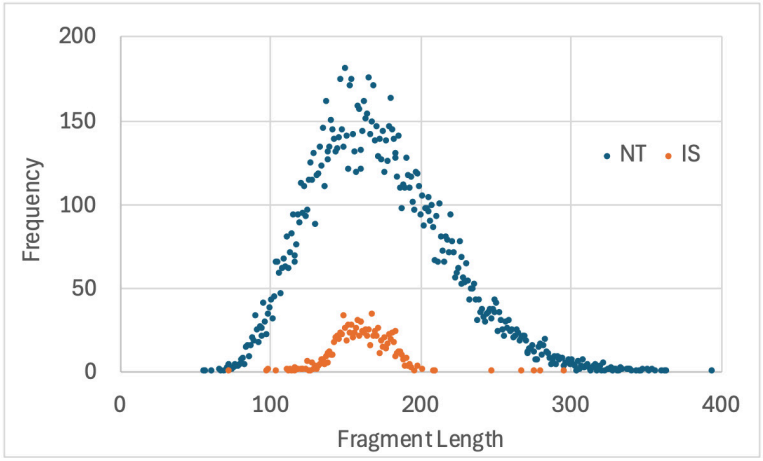

Figure 1: *Read Fragment Size of Internal Standard and Native Template.* Lymphoblast cell line genomic DNA enzymatically fragmented and size selected to simulate 50 ng cfDNA material was mixed with 1,000 copies Accukit ctDNA MRD IS, subjected to Agilent SureSelect XT HS2 library preparation, sequenced on a NextSeq 2000 (200 million reads per sample, 200 paired cycles), error corrected sequencing reads were aligned to a hg19 reference genome appended with the IS FASTA sequences, and a distribution frequency (y-axis) plot was created from read fragments lengths (x-axis) for reads mapping to the regions covered by the IS. IS reads (IS, orange circles) were separated from native template reads (NT, blue circles) based on the unique IS variants.

Table 1: List of mutations present in Accukit™ ctDNA MRD IS, along with the clinically relevant near adjacent mutation.

	Gene	Nucleotide Change	Amino acid	GRCh37 Location	Near Adjacent Mutation
1	AKT1	c.63G>T	p.T21	14:105246537	p.E17K
2	ALK	c.3534G>A	p.N1178	2:29443683	p.F1174L
3	ALK	c.3618G>C	p.S1206	2:29443599	p.G1202R
4	BRAF	c.1806A>T	p.S602	7:140453129	p.V600E
5	EGFR	c.2213_2227del	p.V738_V742del	7:55242443_55242457	p.E746_A750del
6	EGFR	c.2376C>G	p.L792	7:55249078	p.T790M
7	EGFR	c.2562A>C	p.T854	7:55259504	p.L858R
8	KRAS	c.33A>G	p.A11	12:25398286	p.G12C
9	NRAS	c.180T>C	p.G60	1:115256531	p.Q61R
10	PIK3CA	c.3147T>A	p.G1049	3:178952092	p.H1047R

LIMITATIONS OF THE PROCEDURE:

Accukit™ ctDNA MRD IS reagents are not a substitute for control reagents provided with manufactured test kits. Test procedures provided by test kit manufacturers must be followed closely. Consult with manufacturer prior to replacing manufacture test kit controls. Changes to the procedure recommended by the manufacturer may cause unreliable results. This product is offered for Research Use Only. Not for use in diagnostic procedures. The Accukit™ ctDNA MRD IS reagent is not a calibrator and should not be used as such. Unrecommended shipping or storage conditions, or use of expired product could produce erroneous results.

REFERENCES:

1. Siegel JD, Rhinehart E, Jackson M, Chiarello L, and the Healthcare Infection Control Practices Advisory Committee, 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings.

For more information: info@accugenomics.com

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v2.01-2024