AccuGenomics
A higher standard of accuracy

PRODUCT SHEET

Accukit™ Mammo DNA IS

SNAQ™-SEQ Spike-in Standards for Improved CNV

Full Exon Coverage of:
- ATM
- BRCA1
- BRCA2
- PALB2
- (Plus 5 off-target regions as assay normalizers)

Applications include:
- Study breast cancer tumor progression / therapy response
- Optimize workflows for FFPE and cfDNA samples
- Establish analytical accuracy in clinical NGS testing with robust internal quality controls

PRODUCT AND INTENDED USE:

The Accukit™ Mammo DNA IS is an enabling mixture of internal standards (IS) designed to enhance CNV detection in targeted next-generation sequencing (NGS) tests. By integrating IS that biochemically mimic native DNA behavior, this product provides precise quantification and detection of genomic regions associated with breast cancer. Specifically, the product targets exon-level regions of ATM, BRCA1, BRCA2, and PALB2, ensuring the accurate detection of copy number variants (CNVs) and other alterations critical in breast cancer research.

Cat #:	Product Name:	Conc:	Format
2135	Accukit™ Mammo DNA IS	200 copies/uL	1 x 100uL

This product ensures reliable NGS and CNV analysis even under challenging conditions (such as loss of copy, or even degraded FFPE samples) making it a valuable tool to study cancer genomics with greater precision and accuracy.

EXPECTED RESULTS AND INTERPRETATION OF RESULTS:

Accukit™ Mammo DNA IS enables unparalleled accuracy in detecting CNVs by providing:

1. Exon-Level CNV Detection and Precision: Unlike traditional CNV assays, Accukit™ Mammo DNA IS ensures precise exon-specific measurements across BRCA1, BRCA2, ATM, and PALB2, enabling robust detection of both full-gene and partial-exon alterations. Now, targeted library panels can detect CNV with better sensitivity than WGS.

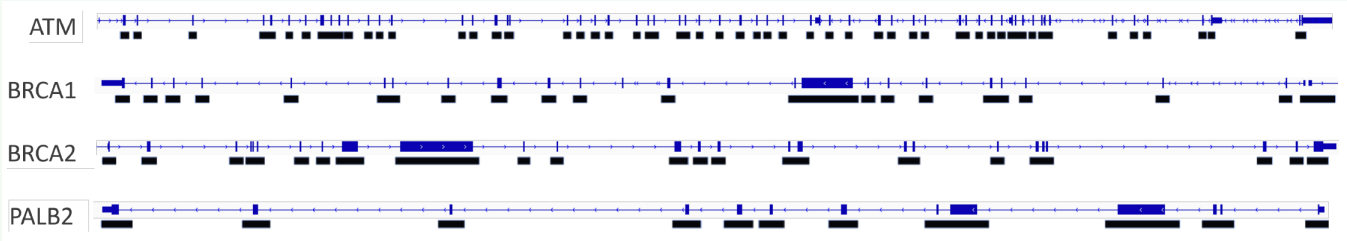
2. Quantitative Genomic Insights: By maintaining a consistent molar ratio with native DNA, SNAQ™-SEQ IS enable reliable quantification of genomic abundance and detection of even subtle copy losses or gains.

3. Reliable Biochemical Integration: Engineered to biochemically covary with native DNA templates, the SNAQ™-SEQ internal standards seamlessly integrate into your NGS workflow, without altering native variant detection. Accukit™ Mammo DNA IS are optimized for use with hybrid capture and amplicon-based sequencing methods to ensure broad applicability.

4. Enhanced Sensitivity & Comprehensive QC: Overcome limitations of conventional CNV detection in targeted libraries using SNAQ™-SEQ to maximize CNV detection for every sample. Integrate precise "per sample" standardization and quality control metrics to improve data quality and reliability.

5. Robust FFPE Performance: Successfully mitigate the challenges of working with degraded or fragmented DNA from FFPE samples, preserving sensitivity and specificity.

Figure 1: Mapping of Accukit™ Mammo DNA IS to Exon Targets.



This detailed mapping highlights the coverage of ATM, BRCA1, BRCA2 and PALB2 exon regions by SNAQ™-SEQ internal standards. Each IS ensures accurate quantification across the genome, allowing for exon-specific CNV detection.

Performance Comparison: Accukit™ Mammo DNA IS outperforms targeted CNV detection workflows (for example, the ThermoFisher OCAv3 panel).

Complete copy number estimates: SNAQ™-SEQ CNV provides results for all genes, as opposed to reporting 50% as "*no results*" from the IonReporter CNV detection pipeline.

- Detection Efficiency: 100% success in detecting exon-level CNVs across all test samples.
- Minimized Errors: Reduced incidence of false positives and negatives compared to conventional methods.
- Enhanced Detection of even Partial Losses: Successfully identifies partial copy losses, which are frequently missed by other systems.

Additional support documents are available by contacting us at info@accugenomics.com.

Accukit™ Mammo DNA IS

Table 1: SNAQ™-SEQ vs. ThermoFisher OCAv3 Pipeline Performance[2]

Comparison of the SNAQ™-SEQ results with OCAv3 original analysis. OCAv3 copy analysis often had insufficient statistics to give a PASSing CNV analysis. Of the 9 overlapping samples, OCAv3 did not give CNV results for half the genes (18 of 36 genes), 4 of which were indicated as copy loss by SNAQ™-SEQ analysis (I-BRCA2, H-BRCA1, L-BRCA1, O-BRCA1), suggesting OCAv3 testing may miss important copy loss detection. SNAQ™-SEQ CNV detection routinely outperformed the OCAv3 pipeline.

SAMPLE	SNAQ™-SEQ	OCAv3
A	BRCA2	(ATM) BRCA1* BRCA2
B	BRCA1 BRCA2	(ATM BRCA1 BRCA2 PALB2)
H[2]	BRCA1[P] BRCA2 PALB2	(BRCA1) BRCA2 PALB2
I	BRCA2	(ATM BRCA1 BRCA2 PALB2)
J	BRCA2	ATM* (BRCA1) BRCA2
K[2]	BRCA1[P] BRCA2	ND
L	BRCA1 PALB2	(BRCA1) PALB2
M	none	(BRCA1 BRCA2 PALB2)
N	none	(BRCA1)
O[1]	BRCA1	(BRCA1 BRCA2)
P	none	ND

KEY
SAMPLE letters correspond to plots in Appendix A
none No copy observed copy loss
ND No Data
* SNAQ™-SEQ results do not support copy loss
GENE indicates loss of copy detected
(GENE) CNV analysis of gene did not pass QC.
[P] partial gene copy number loss
[1] Sample R had insufficient IS added, Sample O was a repeat
[2] Sample H was a repeat of sample K

Further, this simple OCAv3 approach CNV would unlikely detect the partial BRCA1 double copy loss of sample H. Lastly, 2 separate OCAv3 copy loss estimations (samples A and J) were not supported by SNAQ™-SEQ abundance, suggesting OCAv3 method on its own may have indicated and reported false positives.

These data reinforce the superior performance of Accukit™ Mammo DNA IS. (Full results are in the Appendix, Reference 2).

MATERIALS PROVIDED: Accukit™ Mammo DNA IS are available either fragmented (170 +/- 20 bp), or, unfragmented synthetic DNA, depending on your application. The DNA is provided in 10 mM Tris, 0.1 mM EDTA, pH 8.0 buffer.

STORAGE INSTRUCTIONS: Store Accukit™ Mammo DNA 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 MATERIAL INSTABILITY OR DETERIORATION: Accukit™ Mammo DNA 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.

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

INSTRUCTIONS FOR USE: Thaw the Accukit™ Mammo DNA IS vial. Mix to ensure a homogeneous mixture and spin briefly to collect. To minimize stochastic error, the IS should be added to achieve at least 100 unique IS depth. Run a calibration experiment with a few control samples to determine the optimal IS level to add. For example, for 20-30ng from FFPE samples, add enough IS mixture to achieve an IS VAF of 10 to 20%, typically ~ 300 IS copies per sample (or for 30ng cfDNA or fresh tissue, add ~ 700 IS copies). Once the IS input copies have been 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.

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

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

SAFETY PRECAUTIONS: Use Centers for Disease Control and Prevention (CDC) recommended universal precautions for handling reference materials and human specimens. 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™ Mammo DNA IS 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.

LIMITATIONS OF THE PROCEDURE: Accukit™ Mammo DNA IS are not a substitute for control reagents provided with manufactured test kits. Test procedures provided by test kit manufacturers must be followed closely. Changes to the manufacturer's recommended procedure may cause unreliable results. Accukit™ Mammo DNA IS is not a calibrator and should not be used as such. Unrecommended shipping or storage conditions, or use of expired products could produce erroneous results.

REFERENCES:

1. Thomas Morrison, Venus Chirip, Benjamin Yeung, Bradley Austermiller, Bryan Lo; "*The use of Internal Controls in Next Generation Sequencing to improve BRCA1 and BRCA2 gene copy loss detection*", Association for Molecular Pathology Annual Meeting & Expo, Vancouver, BC, November 19-23, 2024.

2. Tom Morrison, Bryan Lo, Venus Chirip; *"SNAQ™-SEQ ThermoFisher OCAv3 CNV Detection at Exon Level, Normal and Tumor Samples"*, Whitepaper, February 16, 2024.

For more information: info@accugenomics.com
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