Advanta Solid Tumor NGS Library Prep Assay

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To advance our understanding of cancer and make strides toward improved patient care, researchers have a growing need to interrogate somatic mutations, often from limited quantities of available samples. Adding to the challenge, translational and clinical researchers in academia and industry settings alike face increasing pressure to maximize laboratory efficiency and productivity by controlling costs and implementing automation to reduce hands-on and turnaround time while ensuring consistent performance and high-quality results.

The Advanta™ Solid Tumor NGS Library Prep Assay with the automated Juno™ system produces targeted amplicon-based barcoded libraries for subsequent analysis on Illumina® NGS platforms. The Advanta Solid Tumor panel is optimized to interrogate a comprehensive selection of high-value, low-variant-frequency somatic mutations within oncology-relevant genes and requires as little as 12.5 ng of DNA starting material per sample. A key advantage of the Advanta Solid Tumor Assay as a component of the Juno workflow is the flexibility to simultaneously process additional oncology panels in the same run, including the Advanta RNA Fusions NGS Library Prep Assay or custom-defined panels, offering maximal efficiency of time, resources and cost for your laboratory.

Highlights

Powerful content–Confidently interrogate SNVs, indels and CNVs with a comprehensive panel of 53 high-value genes to analyze FFPE and fresh-frozen tissue from multiple solid tumor cancers.

High efficiency–Maximize laboratory resources by minimizing hands-on time and costly reagent consumption with nanoliter-scale walkaway automation leveraging Fluidigm microfluidic technology.

Flexible workflow–Streamline NGS library preparation of up to 6 unique assay panels generating actionable insights from both DNA and RNA variants in a single run on the Juno system.

Panel content

Actionable hot spot targets (31 genes)
AKT1, BRAF, CTNNB1, EGFR, ERBB2, ERBB4, FGFR2, FGFR3, FOXL2, GNA11, GNAQ, GNAS, H3F3A, HRAS, IDH1, IDH2, KIT, KRAS, MAP2K1, MAP2K2, MAPK1, MAPK3, MET, MITF, NRAS, PDGFRA, PIK3CA, RAC1, ROS1, SMAD4, TYRP1
CNV targets (24 genes)
AKT1, ALK, BRCA1, BRCA2, CDKN2A, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, HRAS, KIT, MET, NRAS, PDGFRA, PIK3CA, PTEN, RAC1, RB1, RET, RICTOR, TERT, TYRP1, VHL
Full-length coding sequence targets (20 genes)
ALK, BRCA1, BRCA2, CDKN2A, DDR2, HIST1H3B, JAK1, JAK3, NF1, NOTCH1, PALB2, PTEN, RAD51C, RAD51D, RB1, RET, STK11, TERT, TP53, VHL

Figure 1. The Advanta Solid Tumor NGS Library Prep Assay covers 53 genes (total coverage of 234 kb) targeting actionable hot spots, indels and CNVs, and includes 20 genes with full-length coding DNA sequence regions. The genes are associated with numerous solid tumor cancers, including but not limited to breast, colon, liver, lung, melanoma, ovarian and pancreatic. The panel covers ~19,000 pathogenic variants and ~35,000 total variants with COSMIC ID references.

Specifications

Attribute	Specifications
Assays	8 pools (183—197 assays/pool)
Variant types	Somatic analysis of 53 cancer-related genes covering single-nucleotide variants (SNVs) and insertions/deletions (indels) in actionable hot spots from 31 genes, copy number variants (CNVs) from 24 genes and full-length coding DNA sequence of 20 genes.
Technology	Multiplex PCR for targeted, amplicon-based next-generation sequencing (NGS)
Panel size	1,508 amplicons covering 234 kb
Sample types	Human genomic DNA from formalin-fixed, paraffin-embedded (FFPE) or fresh-frozen tissue
gDNA input quantity	≥12.5 ng direct input (for 5% VAF detection)
gDNA input purity	A260/A280 ≥1.5
Library prep time	• Total hands-on time: ~3 hr 30 min • Total library prep time: ~9 hr 30 min
Compatible integrated fluidic circuits (IFCs)	Juno LP 8.8.6 IFC
Samples per run per LP 8.8.6 IFC	Up to 48 samples/run, depending on the number of panels analyzed • Up to 48 samples analyzed with 1 panel (for example, Advanta Solid Tumor panel) • Up to 24 samples analyzed with 2 panels (for example, Advanta Solid Tumor and Advanta RNA Fusions panels)
Samples per kit	Reagents and consumables sufficient for 2 Juno runs; 48 samples/run; 96 samples/kit
Sample barcodes	384 unique barcodes (10 bp) for single-index sequencing
Sequencer	Illumina sequencing systems using 300-cycle chemistry (2 x 150)

Table 1. Overview of the Advanta Solid Tumor NGS Library Prep Assay specifications

Analytical performance results generated from internal validation testing

Variant Class	SNVs (≥5% VAF*)	Indels (<8 bp, >5% VAF)	CNVs
Result	**Average %**		
Positive predictive agreement (sensitivity) true positives/(true positives + false negatives)	98%	99%	98.0%
Positive predictive value true positives/(true positives + false positives)	99%	100%	96.6%
Specificity true negatives/(true negatives + false positives)	99%	100%	100%

Table 2a. Characterized analytical performance of variant detection for the Advanta Solid Tumor NGS Library Prep Assay with controls and reference standards
SNVs: NA12878 and NA24243 Genome in a Bottle (GIAB, curated by the National Institute of Standards and Technology and the Joint Initiative for Metrology in Biology) control sample dilutions were analyzed using 12.5 ng at positions ≥5% expected allele frequency (N=399).
Indels: Horizon OncoSpan Reference Standard was used using 12.5 ng of dilutions based on positions ≥5% expected allele frequency (N=200).
CNVs: Cell line controls (12.5 ng) with known duplications ≥3.5 copies were used to evaluate positive predictive agreement and positive predictive values (N=201). GIAB controls were used to evaluate specificity (N=808).
*Variant allele frequency

Metric	Result
Reads mapping to genome	99%
Reads mapping to target	96%
Limit of detection	5% VAF

Table 2b. Performance for key sequencing metrics demonstrated with NA12878 and NA24243 GIAB samples (N=807) with 12.5 ng of input DNA.

The Juno advantage

The Advanta Solid Tumor NGS Library Prep Assay is optimized to run on the Juno system leveraging the LP 8.8.6 IFC, a microfluidic device approximately the same size and shape as a standard 96-well plate. Sample and assay mixes are dispensed into the IFC, which is subsequently placed on the Juno system for processing. The Juno workflow greatly reduces pipetting steps, enabling walkaway automation by controlling the concurrent and precise pairing of each sample-assay combination within the IFC's parallel, nanoliter-scale reaction chambers that support targeted amplification. When using the Advanta Solid Tumor NGS Library Prep Assay, the Juno workflow can prepare NGS libraries for up to 48 samples in a single run, with approximately 50% less hands-on time than other workflows at similar throughput. Enabling scalability of both sample throughput and content, the Juno automated library preparation workflow delivers cost-effective performance you can trust.

Workflow

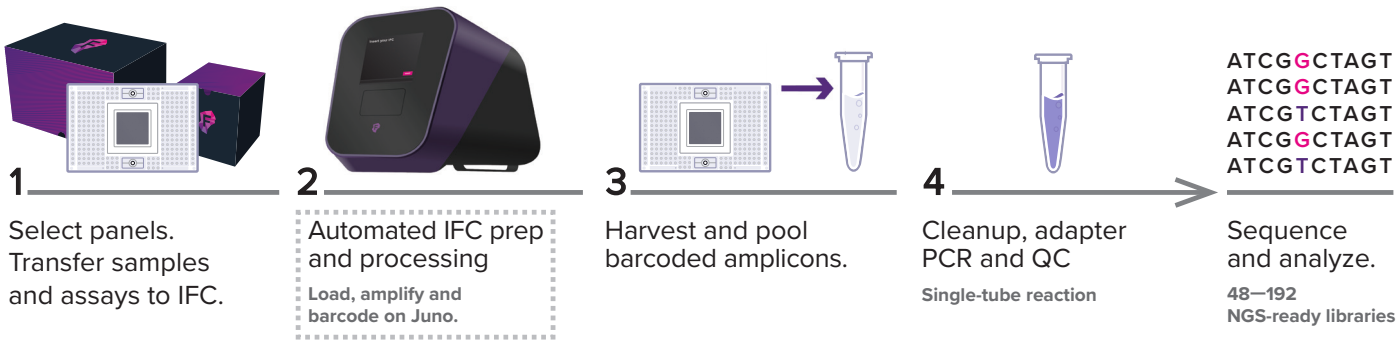

Figure 2. Workflow for the Advanta Solid Tumor NGS Library Prep Assay. Using the LP 8.8.6 IFC and the Juno system, you can configure the run to meet your operating requirements as they change. Choose to process all samples (up to 48) with one panel of assays, such as the Advanta Solid Tumor NGS Library Prep Assay. Alternatively, you can organize the 48 samples into 6 sets of 8 and simultaneously process each sample set using a different assay panel—all within the same IFC run.

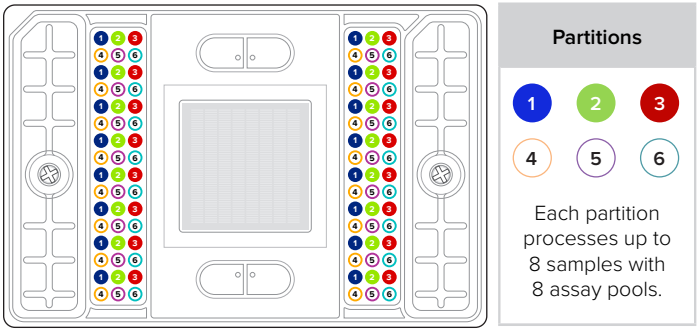

Figure 3. LP 8.8.6 IFC, a unique integrated fluidic circuit enabling content flexibility. The LP 8.8.6 IFC is configured with 6 partitions, each processing 8 samples with 8 assay pools. The partitioned IFC design offers you flexibility in selecting samples and libraries to generate for each run. For example, you may choose to generate libraries from samples with the Advanta Solid Tumor panel exclusively, or choose to include the Advanta RNA Fusions panel in selected partitions. To create a custom panel of selected markers, access the D3™ assay design portal at **fluidigm.com/d3.**

Advanta RNA Fusions NGS Library Prep Assay

Fusion transcripts are an important class of somatic alterations because the resulting chimeric proteins can contribute to oncogene activation or tumor suppressor inactivation.

The Advanta RNA Fusions NGS Library Prep Assay is designed to analyze over 380 gene fusion pairs covering more than 1,000 known breakpoints from both solid tumor and hematologic cancers. Similar to the Advanta Solid Tumor panel, the Advanta RNA Fusions panel is optimized for use with the LP 8.8.6 IFC on the Juno platform. Investigators may process all samples in a given run with the Advanta RNA Fusions assay. Or by taking advantage of the unique partitioning of the LP 8.8.6 IFC, selected samples may be processed simultaneously with the Advanta Solid Tumor panel. The choice is yours, accommodating your specific research needs. Learn more at **fluidigm.com/advanta-rnafusions.**

Product configurations and ordering information

Advanta Solid Tumor NGS Library Prep Assay Materials included support 2 IFC runs, up to 48 samples per run	PN 101-7033
	Quantity
Advanta Solid Tumor NGS Assay Pools (8 Pool Set)	1
Advanta NGS Library Prep Reagent Kit—LP 8.8.6 IFC, 2 IFCs	1
LP 8.8.6 IFC	2
LP 48.48 & 8.8.6 Control Line Fluid	4
LP 48.48 & LP 8.8.6 Barrier Tape	4

Required materials

Targeted DNA Seq Barcode Plates	PN 101-0744
	Number of Advanta Solid Tumor or RNA Fusions Assay kits supported per unit
Set of four 96-well plates; one barcode (20 µL) per well. Total of 384 unique barcodes.	Supports 20 2-IFC kits (40 fully loaded IFCs, or 240 partitions). Sufficient for up to 1,920 samples.

Optional materials

LP 8.8.6 IFC with Accessories Kit Materials included support 2 IFC runs, up to 48 samples/run	PN 101-9226
	Quantity
LP 8.8.6 IFC	2
LP 48.48 & LP 8.8.6 Control Line Fluid	4
LP 48.48 & LP 8.8.6 Barrier Tape	4

Learn more about Fluidigm products at **fluidigm.com/advanta-solidtumor**

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PN 101-9243 B1

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