

Advanta RNA Fusions NGS Library Prep Assay



Highlights

RNA fusion transcripts are an important class of somatic alterations because the resulting chimeric proteins can contribute to oncogene activation or tumor suppressor inactivation. Cancer researchers have a growing need to investigate fusion events, often from limited quantities of available samples. Adding to the challenge, researchers in academia and industry face increasing pressure to maximize laboratory efficiency by controlling costs and implementing automation to reduce hands-on and turnaround time, while ensuring consistent performance and high-quality results.

The Advanta™ RNA Fusions NGS Library Prep Assay and the automated Juno™ system produce targeted amplicon-based libraries for subsequent analysis on Illumina® NGS platforms. The Advanta RNA Fusions panel is optimized to interrogate an extensive selection of high-value gene fusion pairs and requires as little as 10 ng of starting RNA material per sample.

A key advantage of the Advanta RNA Fusions Assay as a component of the Juno workflow is the flexibility to simultaneously process up to six unique oncology panels in the same run. For example, if your study requires the analysis of DNA variants in addition to RNA fusion events, the Advanta Solid Tumor NGS Library Prep Assay or custom-designed panels can be easily co-processed in the same run on the Juno system, delivering maximal efficiency of time, resources and cost management for your laboratory.

Powerful content—Confidently interrogate over 380 gene fusion pairs covering more than 1,000 known break points from both solid tumor and hematologic 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 simultaneously, generating actionable insights from both RNA and DNA variants in a single run on the Juno system.

Representative panel content*

Cancer type	Example of Oncogenic Fusion Genes Covered by the Assay
Breast	BRAF, NOTCH1/2, NTRK3, RAF1
Colon	BRAF, NTRK3, RET, RSPO2, RSPO3
Leukemia	ABL1, PBX1, RARA, RUNX1, RUNX1T1
Lung	ALK, NRG1, NTRK1, RET, ROS1
Melanoma	ALK, BRAF, NTRK1, RAF1, ROS1, RET
Thyroid	ALK, BRAF, NTRK3, RET

Table 1a. Representative selection of cancer types and examples of associated oncogenic fusion genes covered in the Advanta RNA Fusions NGS Library Prep Assay

Gene	Number of Fusion Partners	Gene	Number of Fusion Partners
ABL1	11	NTRK1	13
ALK	26	NTRK3	6
BRAF	29	RET	18
FGFR3	2	ROS1	17
KMT2A	48	RUNX1	15

Table 1b. Representative selection of highly referenced fusion driver genes and the associated number of fusion partners covered in the Advanta RNA Fusions Assay

*For a complete list of genes included in the Advanta RNA Fusions NGS Library Prep Assay, as well as their specific partner genes, learn more at fluidigm.com/advanta-rnafusions.

Specifications

Attribute	Specifications
Assays	Eight pools, average 140 assays per pool
Variant types	RNA fusions
Technology	Multiplex PCR for targeted, amplicon-based next-generation sequencing (NGS)
Targets	Oncology targets: >380 gene fusion pairs covering >1,000 known break points Internal control targets: 79 broadly expressed genes
Sample types	Human total RNA from whole blood, formalin-fixed paraffin-embedded (FFPE) and fresh frozen tissue
RNA input quantity	≥10 ng RNA
Library prep time	<ul style="list-style-type: none"> Total hands-on time: ~3 hr 45 min Total library prep time: ~9 hr 50 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 per run, depending on the number of panels analyzed <ul style="list-style-type: none"> Up to 48 samples processed with 1 panel (for example, Advanta RNA Fusions panel) Up to 24 samples processed with 2 panels (for example, Advanta RNA Fusions and Advanta Solid Tumor panels)
Samples per kit	Reagents and consumables sufficient for 2 runs; 48 samples per run; 96 samples per kit
Sample barcodes	384 unique barcodes (10 bp) for single-index sequencing.
Sequencer	Illumina® sequencing systems using 300-cycle chemistry (2 x 151)

Table 2. Overview of the Advanta RNA Fusions NGS Library Prep Assay specifications and attributes

Analytical performance results generated from internal validation testing

Sample	Number of Replicates	Expected Fusion Events (per Replicate)	Number of True Positives	Number of False Negatives	Sensitivity	Number of True Negatives	Number of False Positives	Specificity	Concordance
Synthetic fusion standards	152	10	1,517	3	99.8%	304	0	100%	98.0%
Cell lines	160	2	318	2	99.4%	2,080	0	100%	98.8%

Table 3. Sensitivity and specificity results observed from control samples with the Advanta RNA Fusions NGS Library Prep Assay.

Seraseq® RNA Fusion Standard v3 (SeraCare Life Sciences) containing 10 known fusion events targeted by the assay and a mix of RNA from H2228 and RS4;11 cell lines [American Type Culture Collection (ATCC)] with 2 known fusion events targeted by the assay were used for this evaluation. Three operators conducted the testing using 2 lots of the Advanta RNA Fusions Assay, 3 lots LP 8.8.6 IFCs and 3 Juno systems. Sensitivity was calculated as the number of true positive fusion events/the total number of known fusion events (true positives + false negatives) when >250 fusion transcript copies are present. Specificity was calculated as the number of true negative calls/the total number of expected negative calls (true negatives + false positives). Concordance is calculated as the number of samples with identical results observed across sensitivity and specificity measures.

The Juno advantage

The Advanta RNA Fusions 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 then 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, which support targeted amplification. With the Advanta RNA Fusions 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.

Relevant content in a flexible library prep workflow



Figure 1. Workflow for the Advanta RNA Fusions 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 RNA Fusions 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.

Advanta Solid Tumor NGS Library Prep Assay

In addition to fusion transcripts, researchers investigate other somatic variant types associated with DNA to deepen their understanding of the mechanisms of cancer.

The Advanta Solid Tumor NGS Library Prep Assay is a comprehensive panel covering single-nucleotide variants (SNVs), insertions/deletions (indels) and copy number variants (CNVs) from 53 high-value genes. Similar to the Advanta RNA Fusions panel, the Advanta Solid Tumor Assay 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 Solid Tumor 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 RNA Fusions Assay. The choice is yours, accommodating your specific research needs. Learn more about the Advanta Solid Tumor Assay at fluidigm.com/advanta-solidtumor.

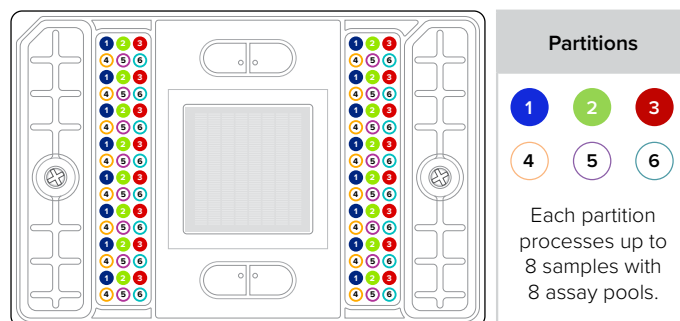


Figure 2. LP 8.8.6 IFC, a unique integrated fluidic circuit enabling content flexibility. The LP 8.8.6 IFC is configured with 6 partitions, each processing 8 samples with 8 assay pools. The partitioned IFC design offers you flexibility when selecting panels to analyze for each run. For example, you may choose to analyze samples with the Advanta RNA Fusions panel exclusively, or choose to include the Advanta Solid Tumor panel in selected partitions. To create a custom panel of selected markers, access the D3™ assay design portal at fluidigm.com/d3.

Product configurations and ordering information

Advanta RNA Fusions NGS Library Prep Assay Materials included support 2 IFC runs, up to 48 samples per run	PN 101-8654
	Quantity
Advanta RNA Fusions NGS Assay Pools (8 Pool Set)	1
Reverse Transcription Reagents (96 Rxns)	1
Advanta NGS Library Prep Reagent Kit—LP 8.8.6 IFC, 2 IFCs	1
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

Required materials

Targeted DNA Seq Barcode Plates	PN 101-0744
	Number of Advanta 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 per 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

Advanta FFPE RNA Extraction Kit (50 extractions)	PN 101-6773
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Learn more about Fluidigm products at fluidigm.com/advanta-rnafusions

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