

Advanta CFTR NGS Library Prep Assay

Frequently Asked Questions

Which CFTR variants does the Advanta CFTR NGS Library Prep Assay cover and how were they chosen?

The Advanta™ CFTR NGS Library Prep Assay was designed to support the sequencing of all 27 exons of the CFTR gene along with select intronic regions. The sequences cover over 250 variants identified as CF-causing by CFTR2.org, which represents an international team of researchers with support from the Cystic Fibrosis Foundation. Refer to any Advanta CFTR NGS Library Prep Assay protocol (see **Table 1**) or the Evidence-Based CFTR Variant Coverage flyer (PN 101-7259) for the list of targeted CFTR variants.

How many amplicons are generated by the Advanta CFTR NGS Library Prep Assay, and what is the size?

The Advanta CFTR NGS Library Prep Assay includes 73 assays organized into eight primer pools. Forty-one assays target SNPs and small indels and generate amplicons with a size range of 196–303 bp. An additional 32 assays target 20 large CFTR exonic deletions and duplications, along with poly_nGT-Tn.

Your CF design only uses a portion of the assay inlets within the IFC. What am I supposed to do with the unused assay inlets?

The open assay inlets may be used to add custom assay content. The Fluidigm D3™ assay design service is available to support you through customized assay design. In the absence of additional custom assay content, the protocol includes instructions for adding an assay blank solution (provided) to the unused assay inlets.

Can I add other variants of interest that are not covered by the Advanta CFTR NGS Library Prep Assay?

Yes. Assays designed for other variants, including those within other genes, may be added to the open assay inlets of the integrated fluidic circuit (IFC). The Advanta CFTR NGS Library Prep Assay uses 8 assay inlets, leaving at least 16 assay inlets open for customization by your facility.

Can I include sample identification markers such as the Fluidigm SNP Trace sample ID panel?

Yes. Assays designed for the SNP Trace™ panel loci may be added to an open assay inlet of the IFC. This will enable the user to match data from the Advanta CFTR NGS Library Prep Assay to a previously determined sample SNP Trace profile, providing an internal verification of sample identity.

How did you handle the detection of the large exonic deletions within your design?

Primers that flank the breakpoints of the large exonic deletions were developed. If the deletion is present, the primers produce a synthetic amplicon that can be detected through sequencing.

Does your product include sample barcodes?

The primary set of sample barcodes is packaged separately from the Advanta CFTR NGS Library Prep Assay due to the product configuration: One unit of the barcodes supports 20 units of the LP 48.48, 2 IFCs kit, or 4 units of the LP 192.24, 10 IFC kit. Your Fluidigm Account Executive can provide ordering information and pricing details at the per sample basis.

The Targeted DNA Seq Barcode Plates (PN 101-0744) product contains a set of four 96-well plates, one unique barcode per well, for a total of 384 unique barcodes.

For dual-index sequencing, the Targeted DNA Seq Library Adapter Set (PN 101-2412) is also needed. This product contains a set of four adapter mixes that supplement 101-0744 to create a total of 1,536 unique barcodes.

How many unique sample barcodes are available?

The primary set of sample barcodes (PN 101-0744) enables the tracking and identification of up to 384 unique samples within a single pool of libraries using single-index chemistry. An optional adapter set (PN 101-2412) is available to support dual indexing, which allows the pooling of up to 1,536 unique samples.

What are the sample requirements?

The Advanta CFTR NGS Library Prep Assay supports human genomic DNA (gDNA) from whole blood, saliva and buccal swabs. The protocol uses 2 µL of gDNA at 30–60 ng/µL, and down to 5 ng of input may be used with an off-IFC preamplification. The gDNA input purity, expressed as A260/A280, is >1.5.

When is the use of preamplification recommended?

Inclusion of an off-IFC preamplification step is recommended when using samples with concentrations below 30 ng/µL or purity <1.5 (A160/A180). Instructions for preamplification are provided as an appendix within the protocol.

How many samples can be processed in a single batch?

Our LP 48.48 IFC can process up to 48 samples in a single run.

Our LP 192.24 IFC can process up to 192 samples in a single run.

Sample libraries from multiple runs may be pooled for multiplex sequencing to further control costs based on the indexing strategy and read depth requirements.

How do I estimate read depth coverage?

Guidelines for determining the estimated read depth are provided within the Advanta CFTR NGS Library Prep Assay protocol.

PN 101-6270: protocol for LP 48.48 IFC on Juno™

PN 101-6957: protocol for LP 48.48 IFC on Access Array™

PN 101-6212: protocol for LP 192.24 IFC on Juno

How many samples can be processed per workday, and what is the total turnaround time from start to finish?

Two IFCs can be processed in a single day by a single operator and a single Juno system when the second IFC is loaded on Juno and run overnight.

Two LP 48.48 IFCs = 96 samples/day

Two LP 192.24 IFCs = 384 samples/day

Greater operating efficiencies are available if multiple library batches are prepared and pooled prior to the cleanup and second adapter PCR. We have customers who routinely generate up to 1,536 NGS-ready libraries during a 5-day workweek.

Can you provide a summary of the assay workflow?

Yes. The assay workflow is summarized in the Advanta CFTR NGS Library Prep Assay protocol appropriate for your IFC and the associated quick reference (see **Table 1**).

What level of skill is required to perform the assay and how complex is the workflow?

Individuals performing the assay should have experience working with molecular protocols that involve PCR and next-generation sequencing (NGS). Otherwise, users should have general lab experience including following good laboratory practices (for example, safety, compliance, training and recordkeeping).

What level of manual input is required?

Most of the workflow is either automated or hands-free (for example, incubations). The Juno system automates the mixture of samples with assays, PCR, barcoding and harvest of amplicons for subsequent processing that results in NGS-ready libraries.

What equipment is required to use the product?

Our product was designed and optimized for use on either of the following Fluidigm systems:

- The Juno system
- The Access Array system

Refer to the system-specific Advanta CFTR NGS Library Prep Assay protocol for a complete list of required equipment (see **Table 1**).

What other assays are available for the Juno system and Access Array system?

The Advanta CFTR NGS Library Prep Assay is the first commercial panel-based product to be launched for the Juno system. Efforts are underway to expand the menu of available panel-based products.

Additional panels are available for the Access Array system. Refer to the Fluidigm website for details.

fluidigm.com: Select **Products > Reagents > Targeted DNA Seq > Standard Panels**.

Fluidigm D3 assay design also offers design services for custom assay development. Refer to the Fluidigm website for details.

d3.fluidigm.com/account/login

Which makes and models of NGS systems are supported by the assay?

The current product is designed and optimized to support use with Illumina® NGS platforms that support 300-cycle sequencing chemistry. Illumina systems include MiSeq™, MiniSeq™, NextSeq™ and HiSeq®.

Can I automate use of the product with my liquid handler?

Yes. Fluidigm IFCs are compatible with a number of commercially available automated liquid handling systems. Contact Fluidigm Technical Support for more specific information on this topic.

What information is available about the performance of the Advanta CFTR NGS Library Prep Assay?

Analytical validation of the Advanta CFTR NGS Library Prep Assay was conducted by Q² Solutions® | EA Genomics, and additional testing was conducted in-house by Fluidigm. Summary data is available describing the results for these studies. Contact Fluidigm Technical Support for more details regarding the analytical validation.

How does this assay compare to competitor assays with regard to performance, cost and workflow, and what sets Fluidigm apart from the competition?

The Advanta CFTR NGS Library Prep Assay was designed to offer the following benefits to the genomics lab:

Time-saving: Achieve consistent results while reducing hands-on time through workflow automation on the Juno system.

Cost-effective: Conserve precious reagents by performing nanoliter-scale reactions using microfluidics technology.

Scalable: Sequence dozens to hundreds of barcoded samples in a single run at high read depth.

These attributes differentiate Fluidigm from the competition.

Do you have any publications from users of the product?

Baylor Genetics is using Fluidigm products, including the Juno system, IFCs and targeted sequencing reagents for a custom CFTR sequencing workflow that has been licensed by Fluidigm.

Can I evaluate the product prior to making a purchase commitment?

Yes. Fluidigm has a program that enables prospective customers to receive one unit of our LP 48.48, 2 IFC kit at no charge to conduct an evaluation.

To receive the no-charge evaluation kit, we require the parties (Fluidigm and the prospective customer's institution) to execute a Material Transfer Agreement (MTA), which describes the terms of use and includes a short questionnaire. A copy of the MTA will be sent to you for review. If acceptable, we can then arrange to execute the MTA and schedule the evaluation.

Contact your Fluidigm Account Executive to discuss the product evaluation process.

How do I order the product?

Contact your Fluidigm Account Executive to discuss the product order process.

What is the cost of the product and what is the effective cost per sample?

Contact your Fluidigm Account Executive to discuss pricing and the effective cost per sample.

What materials are included in each kit?

Each Advanta CFTR NGS Library Prep Assay contains targeted DNA sequencing library reagents (including 4X Master Mix, DNA polymerase and adapter mix), CFTR primers, IFCs and Control Line Fluid.

What materials are needed to use the kit but are not included in the kit?

The following materials, each sold separately, are required to use the Advanta CFTR NGS Library Prep Assay:

Targeted DNA Seq Barcode Plates (PN 101-0744)

Targeted DNA Seq Library Adapter Set (PN 101-2412; required for dual-index sequencing only)

Juno LP 48.48 Barrier Tape—10 Pack (PN 101-2346)

Juno LP 192.24 Barrier Tape—10 Pack (PN 101-1825)

Can Fluidigm sequester specific lots of reagents and/or consumables for my lab, and if so, what is the process for sequestering lots?

Yes. Work with your Fluidigm Account Executive to define your specific needs regarding lot sequester.

What data analysis and bioinformatic pipeline tools are available?

If you already have a pipeline, information regarding the Advanta CFTR NGS Library Prep Assay design can be provided to support pipeline configuration. If you need a pipeline, Fluidigm is vetting commercially available analysis software from various third parties. Contact your Fluidigm Account Executive to discuss requirements.

Have you tested any materials that could be used as controls, and can you recommend a source for control materials?

Materials from Coriell Biorepository and Maine Molecular Quality Controls (MMQCI) were tested as part of the product development and validation. These materials are commercially available and may be suitable for use as controls, but you will need to assess their suitability in accordance with your institution's control policies.

How do I contact Support and what should I expect with regard to service and support of the product?

Contact your Fluidigm Account Executive, or send email to support@fluidigm.com to reach our Support team, which will help direct you to the appropriate contact based on the nature of the support needed.

Alternatively, you can get various types of support through the Fluidigm website portal at fluidigm.com/support:

1. Select **Contact**.
2. Complete and submit the electronic form. Fields with an asterisk (*) are required.

Email

For inquiries regarding sales and ordering, you may directly email salesadmin@fluidigm.com

Phone

Regional numbers are provided for Technical Support and Sales.

Table 1. Reference documents for the Advanta™ CFTR NGS Library Prep Assay based on the product configuration.

Part Number	Document Title	101-6156	101-6158	101-7727	101-7728
		LP 48.48 No Preamp	LP 192.24 No Preamp	LP 48.48 Preamp	LP 192.24 Preamp
101-6270	Advanta CFTR NGS Library Prep on the LP 48.48 IFC with Juno Protocol	✓		✓	
101-6271	Advanta CFTR NGS Library Prep on the LP 48.48 IFC with Juno Quick Reference	✓		✓	
101-6212	Advanta CFTR NGS Library Prep on the LP 192.24 IFC with Juno Protocol		✓		✓
101-6213	Advanta CFTR NGS Library Prep on the LP 192.24 IFC with Juno Quick Reference		✓		✓
101-6957	Advanta CFTR NGS Library Prep on the LP 48.48 IFC with Access Array Protocol	✓		✓	
101-6958	Advanta CFTR NGS Library Prep on the LP 48.48 IFC with Access Array Quick Reference	✓		✓	
101-6273	Advanta CFTR Preamplification Quick Reference			✓	✓
101-7083	Advanta CFTR NGS Preamp Pool Product Insert			✓	✓
101-6272	Advanta CFTR NGS Assay Pools Product Insert	✓	✓	✓	✓
101-7085	Advanta NGS Library Prep Reagent Kit—LP 192.24, 10 IFCs Product Insert		✓		✓
101-7086	Advanta NGS Library Prep Reagent Kit—LP 48.48, 2 IFCs Product Insert	✓		✓	
101-6214	Advanta NGS Preamp Reagent Kit—96 Rxn Product Insert			✓	
101-6215	Advanta NGS Preamp Reagent Kit—960 Rxn Product Insert				✓
100-8765	Bulk Dilution Reagent—25 mL Product Insert				✓
100-6023	Fluidigm Custom Assays SDS	✓	✓	✓	✓
101-7794	Advanta NGS Library Prep Reagent Kit —LP 48.48 SDS	✓		✓	
101-7795	Advanta NGS Library Prep Reagent Kit —LP 192.24 SDS		✓		✓
100-9111	Dilution Reagent SDS			✓	✓

For technical support visit fluidigm.com/support.

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