

NextSeq™ 550Dx High Output Reagent Kit v2.5 (300 Cycles)

FOR IN VITRO DIAGNOSTIC USE

Catalog # 20028871

Intended Use

The Illumina NextSeq 550Dx High Output Reagent Kit v2.5 (300 Cycles) is a set of reagents and consumables intended for sequencing of sample libraries when used with validated assays. The kit is intended for use with the NextSeq 550Dx instrument and analytical software.

Principles of Procedure

The NextSeq 550Dx High Output Reagent Kit v2.5 (300 Cycles) is a specialized single-use set of reagents and consumables for sequencing on the NextSeq 550Dx instrument. For its input, the NextSeq 550Dx High Output Reagent Kit v2.5 (300 Cycles) uses libraries generated from DNA where sample indexes and capture sequences are added to amplified targets. Sample libraries are captured on a flow cell and sequenced on the instrument using sequencing by synthesis (SBS) chemistry. SBS chemistry uses a reversible-terminator method to detect fluorescently-labeled single nucleotide bases as they are incorporated into growing DNA strands. The number of sample libraries depends on the multiplexing supported by the upstream library preparation method.

The package insert *NextSeq 550Dx Instrument* provides instructions for performing sequencing on the NextSeq 550Dx instrument.

Performance characteristics and limitations of the procedure for the NextSeq 550Dx High Output Reagent Kit v2.5 (300 Cycles) were established utilizing the Local Run Manager Germline and Somatic Variant Modules.

Limitations of the Procedure

- 1 For *in vitro* diagnostic use.
- 2 Reads with indels (insertions, deletions, or combinations) where content length is > 25 base pairs (bp) are not aligned by the assay software. Consequently, indels of length > 25 bp are not detectable by the assay software.
- 3 The assay software might not align amplicon reads with extreme content, resulting in the region being reported as wild-type. Such extreme content includes:
 - ▶ Reads containing more than three indels
 - ▶ Reads of length at least 30 bp with single nucleotide variant (SNV) content > 4% of the total amplicon target length (excluding probe regions)
 - ▶ Reads of length < 30 bp with SNV content > 10% of the total amplicon length (including probe regions)
- 4 Large variants, including multi-nucleotide variants (MNVs) and large indels, might be reported as separate smaller variants in the output VCF file.
- 5 Deletion variants can be filtered or missed when spanning two tiled amplicons if the deletion length is greater than or equal to the overlap between the tiled amplicons.
- 6 The system cannot detect indels if they occur directly adjacent to a primer and there is no overlapping amplicon. For regions with overlapping amplicons, the assay cannot detect deletions when the region of

- overlap is smaller than the size of deletion to be detected. For example, if the region of overlap between two adjacent amplicons is two bases, the assay cannot detect any deletions including both of those bases. A single base deletion at either of those bases can be detected.
- 7 As with any hybridization-based library preparation workflow, underlying polymorphisms, mutations, insertions, or deletions in oligonucleotide-binding regions can affect the alleles being probed and, consequently, the calls made during sequencing. For example:
 - ▶ A variant in phase with a variant in the primer region might not be amplified resulting in a false negative.
 - ▶ Variants in the primer region could prevent the amplification of the reference allele, resulting in an incorrect homozygous variant call.
 - ▶ Indel variants in the primer region can cause a false positive call at the end of the read adjacent to the primer.
 - 8 Indels can be filtered due to strand bias if they occur near the end of one read and are soft-clipped during alignment.
 - 9 Small MNVs have not been validated and are only reported in the Somatic Variant Module.
 - 10 Deletions are reported in the VCF at the coordinate of the preceding base per VCF format. Therefore, consider adjacent variants before reporting that an individual base call is homozygous reference.
 - 11 Germline-specific limitations:
 - ▶ The NextSeq 550Dx instrument, using the Local Run Manager Germline Variant Module for NextSeq 550Dx, is designed to deliver qualitative results for germline variant calling (eg, homozygous, heterozygous, wild-type).
 - ▶ When used with the Germline Variant Module, the minimum coverage per amplicon needed for accurate variant calling is 150x. As a result, 150 supporting DNA fragments are required, which is equivalent to 300 overlapping paired-end reads. The number of samples and the total number of bases targeted affect coverage. GC-content and other genomic content can affect coverage.
 - ▶ Copy number variation can affect whether a variant is identified as homozygous or heterozygous.
 - ▶ Variants in certain repetitive context are filtered out in the VCF files. The RMxN repeat filter is used to filter variants if all or part of the variant sequence is present repeatedly in the reference genome adjacent to the variant position. For germline variant calling, at least nine repeats in the reference are required for a variant to be filtered, and only repeats with length up to 5 bp are considered (R5x9).
 - ▶ An indel and an SNV at a single locus can result in only one variant being reported.
 - 12 Somatic-specific limitations:
 - ▶ The NextSeq 550Dx instrument, using the Local Run Manager Somatic Variant Module for NextSeq 550Dx, is designed to deliver qualitative results for somatic variant calling (eg, presence of a somatic variant with a variant frequency ≥ 0.026 with a limit of detection of 0.05).
 - ▶ When used with the Somatic Variant Module, the minimum coverage per amplicon needed for accurate variant calling is 450x per oligonucleotide pool. As a result, 450 supporting DNA fragments are required per oligonucleotide pool, which is equivalent to 900 overlapping paired-end reads. The number of samples and the total number of bases targeted affect coverage. GC-content and other genomic content can affect coverage.
 - ▶ For somatic variant calling, at least six repeats in the reference are required for the variant to be filtered, and only repeats with length up to 3 bp are considered (R3x6).
 - ▶ The Somatic Variant Module cannot differentiate between germline and somatic variants. The module is designed to detect variants across a range of variant frequencies, but variant frequency cannot be used to differentiate somatic variants from germline variants.
 - ▶ Normal tissue in the specimen impacts the detection of variants. The reported limit of detection is based on a variant frequency relative to the total DNA extracted from both tumor and normal tissue.

Reagent Kit Components

Each component of the NextSeq 550Dx High Output Reagent Kit v2.5 (300 Cycles) is provided in a separate box. Promptly store components at the indicated temperature to ensure proper performance. The following is a list of reagent kit components.

Table 1 Reagent Kit Components

Component	Quantity	Fill Volume	Description	Storage*
NextSeq 550Dx High Output Reagent Cartridge v2 (300 cycles)	1 each	Various	Clustering and sequencing reagents	-25°C to -15°C
NextSeq 550Dx Buffer Cartridge v2 (300 cycles)	1 each	Various	Buffers and wash solution	15°C to 30°C
NextSeq 550Dx High Output Flow Cell Cartridge v2.5 (300 cycles)	1 each	N/A	Single-use, paired-end, glass flow cell	2°C to 8°C
NextSeq 550Dx Accessory Box (300 cycles)	1 tube	12 ml	Library dilution buffer	-25°C to -15°C

*The NextSeq 550Dx High Output Flow Cell Cartridge v2.5 (300 cycles) is shipped at ambient temperature.

Lot Numbers

The reagent kit has a single lot number, which is referred to as the reagent kit lot number. Each box in the reagent kit is printed with the reagent kit lot number. Reagent kit components that are inside the boxes are printed with component-specific lot numbers that are different from the reagent kit lot number. Keep sequencing consumables stored in their boxes until ready for use to maintain kit lot association. See the Certificate of Analysis of the reagent kit for details about reagent part numbers and lot numbers.

Storage and Handling

- 1 Room temperature is defined as 15°C to 30°C.
- 2 Reagent kit components are stable when stored at the indicated storage temperatures until the expiration date on the label.
- 3 The NextSeq 550Dx Accessory Box and NextSeq 550Dx Reagent Cartridge are stable for a maximum of one thaw to room temperature before the specified expiration date. The reagent cartridge is stable for up to 6 hours when thawed in a room temperature water bath. Alternatively, the reagent cartridge may be thawed at 2°C to 8°C for up to 5 days before use.
- 4 Changes in the physical appearance of the reagents can indicate deterioration of the materials. If changes in the physical appearance occur after mixing, such as obvious changes in reagent color, or cloudiness apparent with microbial contamination, do not use the reagents.

Equipment and Materials Required, Sold Separately

- ▶ NextSeq 550Dx Instrument, Catalog # 20005715

Warnings and Precautions



CAUTION

Federal law restricts this device to sale by or on the order of a physician or other practitioner licensed by the law of the State in which he/she practices, to use or order the use of the device.

- 1 The NextSeq 550Dx High Output Reagent Kit v2.5 (300 Cycles) contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations.
- 2 For environmental, health, and safety information, see the safety data sheet (SDS) at support.illumina.com/sds.html.

- 3 Immediately report any serious incidents related to this product to Illumina and the Competent Authorities of the member states in which the user and the patient are established.
- 4 Failure to follow the procedures as outlined may result in erroneous results or significant reduction in sample quality.
- 5 Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink, or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and assay reagents. Wash hands thoroughly after handling specimens and assay reagents.
- 6 Proper laboratory practices and good laboratory hygiene are required to prevent PCR products from contaminating reagents, instrumentation, and genomic DNA samples. PCR contamination may cause inaccurate and unreliable results.
- 7 To prevent contamination, ensure that pre-amplification and post-amplification areas have dedicated equipment (eg, pipettes, pipette tips, vortexer, and centrifuge).

Instructions for Use

See the package insert *NextSeq 550Dx Instrument* and applicable reference guides.

Performance Characteristics

See the package insert *NextSeq 550Dx Instrument*.

Revision History

Document	Date	Description of Change
Document # 1000000073349 v03	November 2021	Added Warnings and Precautions statement around reporting serious incidents. Added Revision History table.

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