Data Sheet

Agilent
Trusted Answers

SureSelect XT HS2 DNA Reagent Kit

Because flexibility and performance matter

Key Features:

- Generate high-quality libraries with as little as 10 ng of DNA input from intact or highly degraded FFPE samples.
- Choose between mechanical or enzymatic shearing workflows based on your needs.
- Minimize index hopping and maximize throughput with 384 unique dual sample indexing (UDI).
- Use duplex molecular barcodes (duplex MBC) to better suppress false positives and more accurately detect low variant allele frequency (VAF).
- Order everything you need for next generation sequencing (NGS) library preparation and target enrichment, including beads, from one vendor.

Overview

The Agilent SureSelect XT HS2 DNA reagent kit is a state-of-the-art NGS library preparation and target enrichment solution. It provides a streamlined and flexible workflow, excellent performance, and comprehensive features that can be used in various NGS applications (Figure 1). The kit offers a complete solution designed to satisfy your NGS library preparation needs. The workflow is optimized for FFPE samples which are essential for cancer research. Up to 384 unique dual sample indexing enables high-throughput labs to process and sequence hundreds of samples simultaneously. The ability to generate consensus calls using MBC information from both strands (duplex MBC) significantly improves the accuracy of low VAF detection which is critical in liquid biopsy applications. Agilent also offers a one-stop shop experience, including beads, quality control, automation, and analysis solutions.

Sample	Library Preparation & Target Enrichment
10 - 200 ng DNA input	Mechanical/enzymatic shearing
	384 unique dual sample indexing
FFPE and high-quality samples	Single/duplex MBC
	1-day/2-day workflow
	Beads included option
	Bravo automation

Figure 1. The SureSelect XT HS2 DNA reagent kit offers one streamlined workflow with high flexibility and comprehensive features to satisfy your NGS library preparation needs.

One solution for your NGS library preparation needs

The highly flexible workflow and comprehensive features of the SureSelect XT HS2 DNA reagent kit enable users to choose the workflow that best satisfies their needs. The kit is optimized for a wide range of DNA input (10–200 ng), various sample types (intact or FFPE samples), different shearing methods (mechanical vs. enzymatic) and sequencing chemistries (2 x 100 bp or 2 x 150 bp). The users can take advantage of the 90-minute fast hybridization to generate enriched libraries in a single day. They also have the flexibility to split the workflow into two days. Up to 384 unique dual sample indexing enables users to multiplex hundreds of samples without worrying about index hopping. Duplex MBC enables users to filter out artifacts generated by PCR amplification during the library preparation process. It significantly reduces the number of false positive calls and improves the accuracy of low VAF detection. It's worth noting that both 384 UDIs and duplex MBC can be utilized simultaneously, an improvement over the existing XT Low Input products.

The kit is configured with two options—with and without the Ampure and Streptavidin beads. Users don't need to go to a third party vendor and can purchase everything they need for NGS library preparation, from upfront quality control to downstream analysis, from Agilent. The Agilent SureSelect XT HS2 DNA starter kit (G9982A) simplifies your ordering experience even further. You get everything you need for NGS library preparation, including the reagent kit, enzymatic fragmentation module, and the beads, by ordering a single part number. The SureSelect XT HS2 DNA reagent kit is also compatible with Agilent's Bravo automation system, and will be enabled on the fully automated Magnis automation system in the near future.

Superior performance of the SureSelect XT HS2 DNA reagent kit

Conversion efficiency is a key metric to evaluate library preparation performance. It measures the percentage of input DNA being converted into a fully adaptor ligated library. Better conversion efficiency suggests that more DNA molecules are sequenceable in the final library which is measured by library complexity. Higher library complexity indicates that there is less chance to sequence the same DNA molecules over and over again. Therefore, it is more likely to get higher base coverage when sequencing depth is increased. This is critical for low VAF detection in tumor and liquid biopsy samples. The SureSelect XT HS2 DNA reagent kit shows better conversion efficiency compared to the competition using both fresh frozen and FFPE samples (Figure 2). For basic sequencing metrics, duplication rate, uniformity and 100X base coverage are comparable among the three kits. However, the SureSelect XT HS2 DNA reagent kit shows significantly higher on target %, 300X base coverage and library complexity compared to the competitors (Figures 3 and 4). In particular, with 10 ng of DNA input and 1,000X raw sequencing, the 300X base coverage of the SureSelect XT HS2 DNA reagent kit (53.3%) is dramatically higher than the other two kits (9.4% and 6.2% respectively).

Duplex MBC significantly improves detection accuracy of low VAF

Short and random DNA nucleotides called molecular barcodes are incorporated into the NGS library during the adaptor ligation step. These unique sequences can be used as identifiers for individual DNA molecules to filter out false positive calls and provide a more accurate variant calling result. This feature is essential for detecting variants with low allele frequencies in heterogeneous samples, such as tumor biopsy or ctDNA samples.

For the SureSelect XT HS2 DNA reagent kit, you can generate consensus calls using MBC information from both strands (duplex MBC), single strand (single MBC) or discard MBC information (no MBC), based on the application. While both duplex and single MBC improve the accuracy of low VAF detection compared to no MBC, duplex MBC enables the most effective error correction. The number of false positive calls of low VAF (=<4% in this analysis) for duplex MBC were reduced significantly by 74% and 93% compared to single MBC and no MBC, respectively (Figure 5).

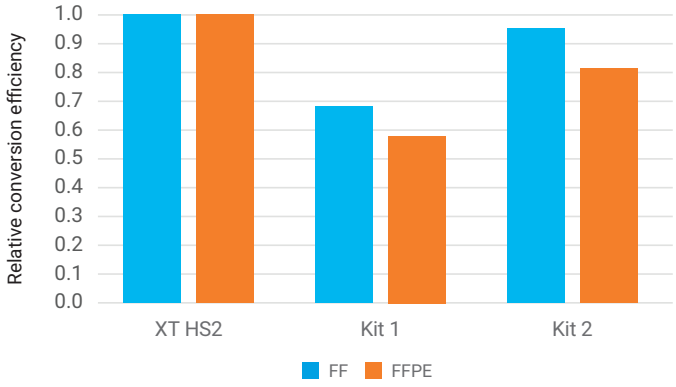

Figure 2. Improved conversion efficiency for both fresh frozen and FFPE samples. 10 ng of DNA from lung tumor fresh frozen and normal kidney FFPE (ddCq=1.8, DIN=3.2) samples served as the input for library preparation using the SureSelect XT HS2 DNA reagent kit and two competing kits (Kit 1 and Kit 2). For Kit 1 and Kit 2, libraries were prepared using library preparation kit from vendor K and different adaptors from vendor ID. Sample quality was determined by the Agilent TapeStation (DIN) and Agilent NGS FFPE QC kit (ddCq value). Limited PCR cycles were performed to ensure linear amplification. Relative conversion efficiency was calculated using XT HS2 as reference.

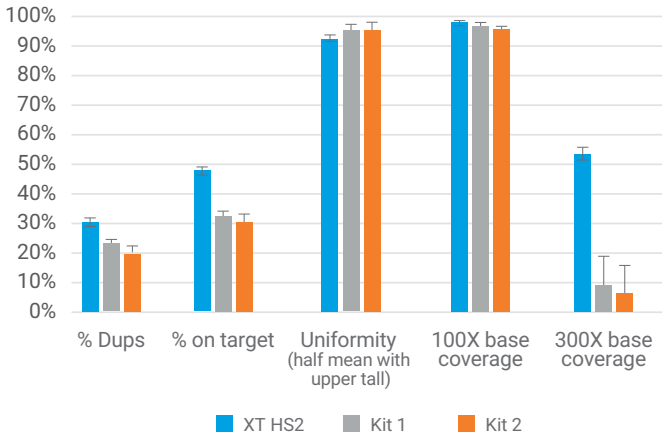

Figure 3. Superior 300X base coverage with 10 ng DNA input, compared to competitors' kits. 10 ng of HapMap sample (NA12878) was used as input. For XT HS2, library construction and target enrichment were performed using the SureSelect XT HS2 DNA reagent kit and a 25 kb custom probe from Agilent. For Kit 1 and Kit 2, libraries were prepared using library preparation kit from vendor K and different adaptors from vendor ID. Target enrichment was performed using a 25 kb custom probe from vendor ID for both Kit 1 and Kit 2. Libraries were sequenced (2 x 100 bp) on the Illumina MiSeq system. Reads were mapped to hg19, normalized to 1,000X raw sequencing depth.

Figure 4. Significantly higher library complexity compared to the competitors' kits. 10 ng of HapMap sample (NA12878) was used as input. For XT HS2, library construction and target enrichment were performed using the SureSelect XT HS2 DNA reagent kit and a 25 kb custom probe from Agilent. For Kit 1 and Kit 2, libraries were prepared using library preparation kit from vendor K and different adaptors from vendor ID. Target enrichment was performed using a 25 kb custom probe from vendor ID for both Kit 1 and Kit 2. Libraries were sequenced (2 x 100 bp) on the Illumina MiSeq system. Reads were mapped to hg19, normalized to 1,000X raw sequencing depth and HS library size was then determined.

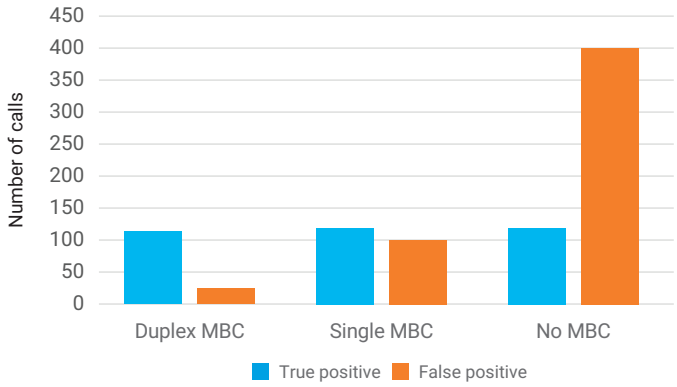

Figure 5. Duplex MBC significantly lower false positive calls for low VAF. 20 ng of HapMap sample mixture (98% NA12878 and 2% NA24385) was used as input. Library construction and target enrichment were performed using the SureSelect XT HS2 DNA reagent kit and Agilent SureSelect Cancer All-In-One Lung assay. Libraries were sequenced (2 x 100 bp) on the Illumina HiSeq 4000 system. Reads were mapped to hg19, normalized to 10,000X raw sequencing depth. The Trimmer and LocatIt tools in the Agilent Genomics Toolkit (AGeNT) were used to process MBCs and generate consensus reads for each MBC family. Duplex MBC uses MBC sequences from both strands to generate consensus variant calls, while Single MBC creates consensus reads without taking the strand information into account. Variants with allele frequencies equal or lower than 4% were selected for this analysis.

Ordering information

Product	Part Number
SureSelect XT HS2 DNA reagent kit with index primer pairs 1–16, 16 reactions	G9981A
SureSelect XT HS2 DNA starter kit with index primer pairs 1–16, 16 reactions	G9982A
SureSelect XT HS2 DNA reagent kit with index primer pairs 1–96, 96 reactions	G9983A
SureSelect XT HS2 DNA reagent kit with index primer pairs 97–192, 96 reactions	G9983B
SureSelect XT HS2 DNA reagent kit with index primer pairs 193–288, 96 reactions	G9983C
SureSelect XT HS2 DNA reagent kit with index primer pairs 289–384, 96 reactions	G9983D
SureSelect XT HS2 DNA reagent kit with AMPure XP/Streptavidin beads and index primer pairs 1–96, 96 reactions	G9984A
SureSelect XT HS2 DNA reagent kit with AMPure XP/Streptavidin beads and index primer pairs 97–192, 96 reactions	G9984B
SureSelect XT HS2 DNA reagent kit with AMPure XP/Streptavidin beads and index primer pairs 193–288, 96 reactions	G9984C
SureSelect XT HS2 DNA reagent kit with AMPure XP/Streptavidin beads and index primer pairs 289–384, 96 reactions	G9984D
SureSelect XT HS2 DNA library preparation kit with index primer pairs 1–96, 96 reactions	G9985A
SureSelect XT HS2 DNA library preparation kit with index primer pairs 97–192, 96 reactions	G9985B
SureSelect XT HS2 DNA library preparation kit with index primer pairs 193–288, 96 reactions	G9985C
SureSelect XT HS2 DNA library preparation kit with index primer pairs 289–384, 96 reactions	G9985D
SureSelect XT HS2 DNA target enrichment kit, 12 hybridizations	G9987A
SureSelect enzymatic fragmentation kit, 16 reactions	5191-4079
SureSelect enzymatic fragmentation kit, 96 reactions	5191-4080

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