

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 formalin-fixed, paraffin-embedded (FFPE) samples
- Choose between mechanical or enzymatic shearing workflow based on your needs
- Minimize index hopping and maximize throughput with 384 UDI
- Use duplex MBC to better suppress false positives and more accurately detect low VAF
- Use optional MBC-free adaptors for germline applications and maximum sequencing yield
- Leverage high flexibility with two hybridization workflow options
- Order everything you need for 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 next-generation sequencing (NGS) library preparation and target enrichment solution. It provides a streamlined and flexible workflow, excellent performance, and comprehensive features suitable for various NGS applications (Figure 1). The kit offers a complete solution designed to meet your NGS library preparation needs. The workflow ensures reliable and efficient processing of FFPE samples, which is essential for cancer research.

With up to 384 unique dual sample indexing (UDI), high-throughput labs can 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 variant allele frequency (VAF) detection, which is critical in liquid biopsy applications. Additionally, the use of MBC-free adaptors provides additional sequencing output for germline applications. Fast and overnight hybridization workflow options enhance flexibility for your work shift. Agilent also offers a one-stop shop experience, including beads, quality control, automation solutions.

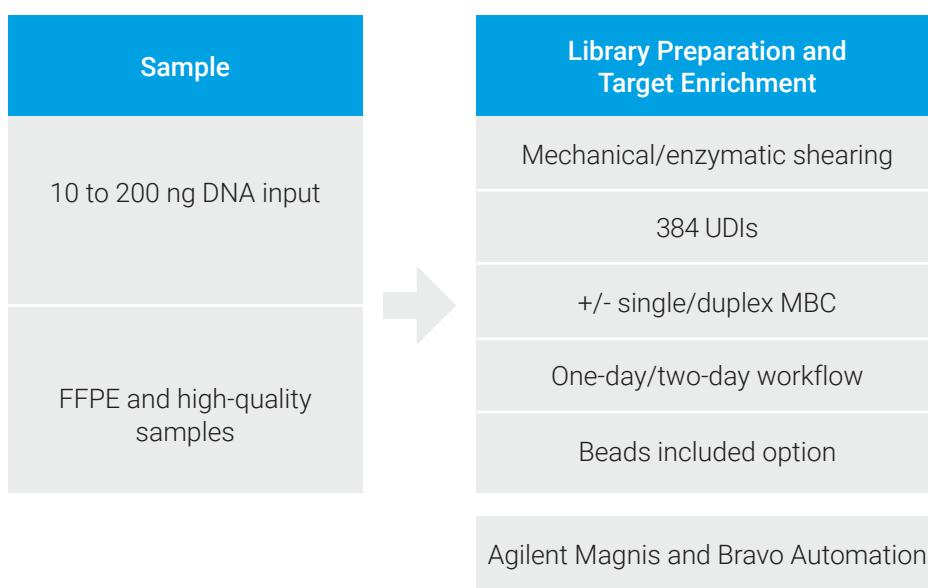


Figure 1. The SureSelect XT HS2 DNA reagent kit offers one streamlined workflow with high flexibility and comprehensive features to satisfy your NGS library preparation needs.

One solution for your NGS library preparation needs

The highly flexible workflow and comprehensive features of the SureSelect XT HS2 DNA reagent kit enable a choice of workflow that best satisfies your needs. The kit is optimized for a wide range of DNA input (10 to 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). Take advantage of the 90-minute fast hybridization to generate enriched libraries in a single day, while also having the flexibility to split the workflow into two days with an overnight hold. The 16-hour overnight hybridization is another option to enrich libraries in two days, without the need to redesign probes. With up to 384 unique dual sample indexing, you can multiplex hundreds of samples without worrying about index hopping. Duplex MBC allows for the filtering out of 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. Notably, that both 384 UDIs and duplex MBC can be utilized simultaneously, which is an improvement over the existing XT low input products.

The SureSelect XT HS2 DNA reagent kit gives an additional workflow flexibility, with MBC-tagged or MBC-free library prep kits that can be used in conjunction with either the standard or the overnight hybridization target enrichment kit.

The kit is configured with two options—with and without the Ampure and Streptavidin beads. Purchase everything you need for NGS library preparation, from upfront quality control to downstream sample processing on a fully automated system, from Agilent. The Agilent SureSelect XT HS2 DNA starter kit (G9982A) simplifies your ordering experience by providing everything you need for NGS library preparation, including the reagent kit, enzymatic fragmentation module, and the beads, with a single part number. The SureSelect XT HS2 DNA reagent kit works with both Agilent Bravo NGS workstation and Agilent Magnis NGS prep system.

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 of sequencing the same DNA molecules repeatedly. Therefore, it is more likely to achieve 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 products from other suppliers using both fresh frozen and FFPE samples (Figure 2).

Basic sequencing metrics, including duplication rate, uniformity, and 100X base coverage, were comparable among the three kits. However, the SureSelect XT HS2 DNA reagent kit shows significantly higher on-target %, 300X base coverage, and library complexity (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

Optional short and random DNA nucleotides called molecular barcodes can be incorporated into the NGS library during the adaptor ligation step. These unique sequences serve 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 ($\leq 4\%$ in this analysis) for duplex MBC were reduced significantly by 74% and 93% compared to single MBC and no MBC, respectively (Figure 5).

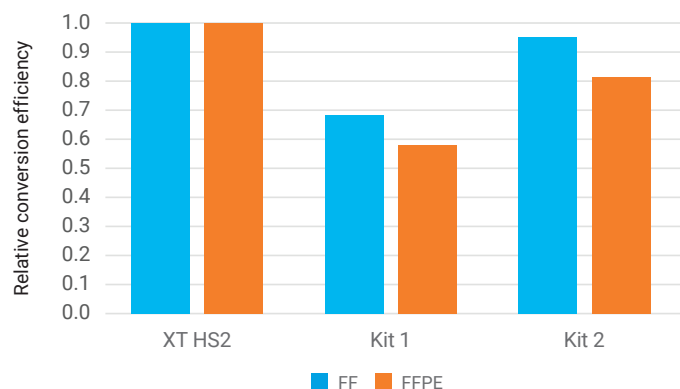


Figure 2. Improved conversion efficiency for both fresh frozen (FF) and FFPE samples. 10 ng of DNA from lung tumor FF and normal kidney FFPE ((ddCq=1.8, DIN=3.2) samples served as the input for library preparation using the SureSelect XT HS2 DNA reagent kit and two competing kits (Kit 1 and Kit 2). For Kit 1 and Kit 2, libraries were prepared using a library preparation kit from vendor A and different adaptors from vendor B. 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.

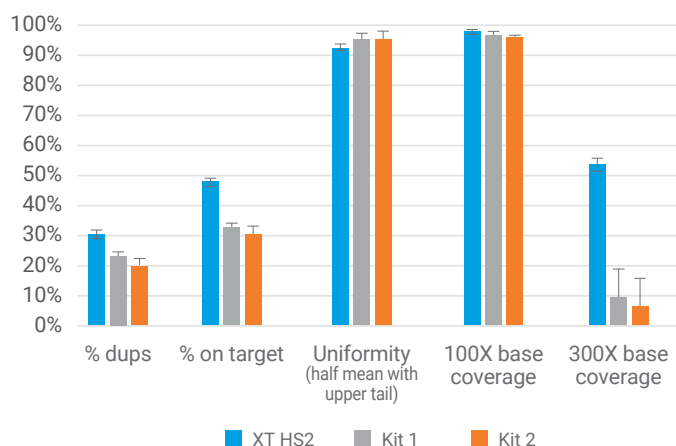


Figure 3. Superior 300X base coverage with 10 ng DNA input of HapMap sample (NA12878). For XT HS2, library construction and target enrichment were performed using the SureSelect XT HS2 DNA reagent kit and a 25 kb custom probe from Agilent. For Kit 1 and Kit 2, libraries were prepared using library preparation kit from vendor A and different adaptors from vendor B. Target enrichment was performed using a 25 kb custom probe from vendor B 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.

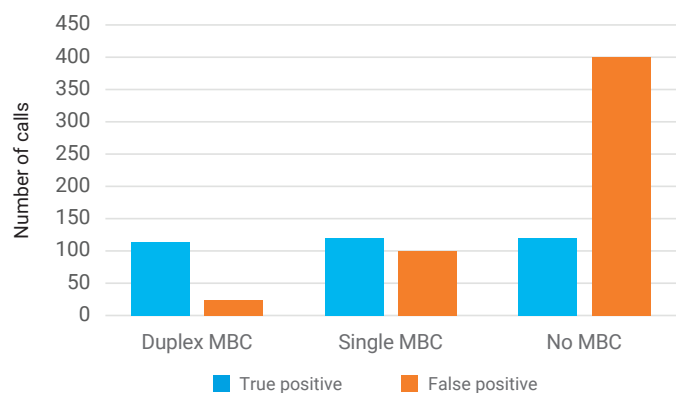


Figure 5. Duplex MBC significantly lower false positive calls from low VAF. 20 ng of HapMap sample mixture (98% NA12878 and 2% NA24385) was used as input. Library construction and target enrichment were performed using the SureSelect XT HS2 DNA reagent kit and Agilent SureSelect Cancer All-In-One Lung assay. Libraries were sequenced (2 x 100 bp) on the Illumina MiSeq system. The Trimer 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 to or lower than 4% were selected for this analysis.

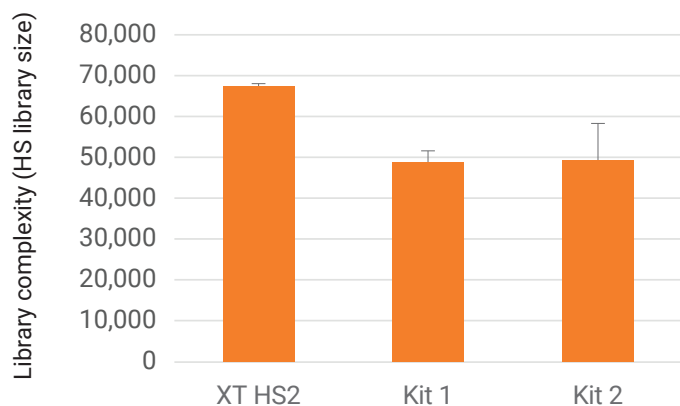


Figure 4. Significantly higher library complexity. 10 ng of HapMap sample (NA12878) was used as input. For XT HS2, library construction and target enrichment were performed using the SureSelect XT HS2 DNA reagent kit and a 25 kb custom probe from Agilent. For Kit 1 and Kit 2, libraries were prepared using library preparation kit from vendor A and different adaptors from vendor B. Target enrichment was performed using a 25 kb custom probe from vendor B 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.

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 library preparation kit with MBC-free adaptors and index primer pairs 1-96, 96 reactions	G9956A
SureSelect XT HS2 library preparation kit with MBC-free adaptors and index primer pairs, 97-192, 96 reactions	G9956B
SureSelect XT HS2 library preparation kit with MBC-free adaptors and index primer pairs, 193-288, 96 reactions	G9956C
SureSelect XT HS2 library preparation kit with MBC-free adaptors and index primer pairs, 289-384, 96 reactions	G9956D
SureSelect XT HS2 DNA target enrichment kit, 12 hybridizations	G9987A
SureSelect XT HS2 target enrichment kit, 96 hybridizations	G9987B
SureSelect XT HS2 target enrichment kit, overnight hybridization, 12 hybridizations	G9957A
SureSelect XT HS2 target enrichment kit, overnight hybridization, 96 hybridizations	G9957B
SureSelect enzymatic fragmentation kit, 16 reactions	5191-4079
SureSelect enzymatic fragmentation kit, 96 reactions	5191-4080

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