Data Sheet

Agilent
Trusted Answers

SureSelect XT HS2 RNA Reagent Kit

One easy, parallel workflow for RNA and DNA-seq to simplify your work

Key features

- Parallel RNA/DNA workflow enabled by a modular configuration
- High-quality, strand-specific libraries from low inputs of total RNA (including FFPE samples)
- Streamlined workflow with a short turnaround time
- Excellent performance with high library complexity and a low duplication rate
- 384 unique dual indexes (UDI) to maximize multiplexity and minimize index hopping
- Molecular barcodes (MBC) for accurate gene expression
- Compatible with SureSelect exome panels or custom NGS panels created with the SureDesign software

Overview

The Agilent SureSelect XT HS2 RNA Reagent Kit is an advanced solution for targeted RNA-Seq. It is optimized for low-input, formalin-fixed paraffin-embedded (FFPE) samples, a critical sample type for translational cancer research. Its streamlined workflow and 90-minute hybridization results in one of the fastest turnaround times on the market (1 to 2 days). 384 UDIs minimize index hopping and allow researchers to multiplex hundreds of samples in a single sequencing run to reduce costs.

The SureSelect XT HS2 RNA Reagent kit's robust performance and high library complexity also enable higher sensitivity for detecting low-expression genes. The incorporated MBCs allow the removal of PCR duplicates (while keeping non-PCR duplicate reads) and provide more consistent, accurate gene expression data (particularly for low-input samples). Finally, our fully integrated portfolio—including beads, quality control, automation, and analysis solutions—helps you avoid the anxiety of dealing with multiple vendors for ordering and support.

A parallel workflow to simplify your day

The modular design of the SureSelect XT HS2 RNA Reagent Kit gives you maximum flexibility for your NGS protocols. From the adaptor ligation step onward, the workflow is parallel to its DNA counterpart, the SureSelect XT HS2 DNA Reagent kit (Figure 1A), for a single, parallel workflow. Since both kits are built upon the same XT HS2 chemistry, you do not need to optimize different kits for various sample types and inputs. In addition to SureSelect targeted RNA-seq, we also offer the SureSelect mRNA Library Preparation kit for poly-A selection library preparation This kit offers the same modular design as the XT HS2 kit to give you a streamlined and flexible workflow (Figure 1B).

Focus on what matters the most

RNA is the next frontier for biomarker discovery, and FFPE tissue is an important sample type in functional validation studies. However, the FFPE fixation process causes significant degradation to RNA quality and makes whole transcriptome and/or mRNA-Seq analyses more challenging.

Targeted RNA-Seq, however, offers an effective and economic way to retrieve information from these difficult samples. This method uses hybridization probes that are specific to target genes and capture only genes relevant to your interest. It yields a significantly higher percentage of reads that map to the coding regions of genes of interest (Figure 2) and maintains an excellent overall correlation compared to whole transcriptome and mRNA-Seq (Table 1). By focusing these reads on specific regions, you can achieve deeper coverage with higher sensitivity for fusion detection (Figure 3).

Excellent performance of the SureSelect XT HS2 RNA Reagent Kit

Library complexity is a key metric for evaluating the quality of your RNA-Seq library. Higher library complexity suggests that more RNA molecules were captured, which increases accuracy for gene expression and RNA fusion analyses. High-complexity libraries also have a higher chance to detect low expressors, a feature critical for applications such as immuno-oncology.

The SureSelect XT HS2 RNA Reagent kit shows significantly higher library complexity compared to libraries prepared by a competitor's kit in both intact and FFPE samples (Figure 4a). Lower rates of PCR duplication (Figure 4b) and rRNA carry over (Figure 4c) indicate that more sequencing reads are maintained for downstream analysis by greater filtration of non-informative reads. The high percentage of exonic regions and low percentage of intronic regions are consistent with the high hybrid capture efficiency of the SureSelect XT HS2 RNA Reagent Kit (Figure 4d).

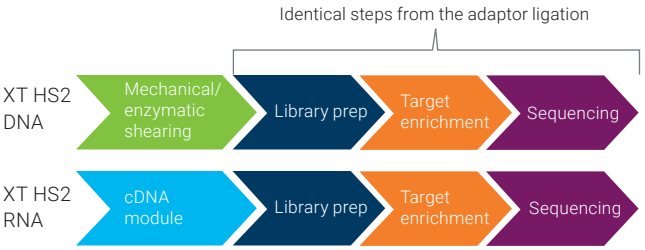
Deduplication is an important step in the analysis of low-input and/or deep-coverage RNA-Seq libraries. Compared to standard deduplication methods using chromosomal coordinates, deduplication with molecular barcodes (MBCs) prevents the removal of non-PCR duplicates for more accurate data. Figures 4a and 4b demonstrate that deduplication with MBC information significantly lowers duplication rate and improves library complexity compared to deduplication by chromosomal coordinates.

Accurate gene expression profiling using MBC information

MBCs are short, random sequences of DNA that are incorporated into the NGS library during the ligation step. They act as a powerful tool for the QC of NGS data: if two or more reads have the same MBC, the duplicates can be excluded to provide you with more reliable, sensitive results.

MBCs have been widely used in DNA sequencing to provide more accurate variant calling results, particularly for variants with low allele frequencies. While the use of MBCs in RNA-Seq experiments is still relatively new, there is a growing body of evidence that indicates MBCs can improve gene expression accuracy by eliminating real PCR duplicates. This feature is even more critical for low-input and/or low-quality samples.

Unlike deduplication using only genomic coordinates (which can remove valid reads that share sequences), deduplication by MBC only removes true PCR duplicates. This reduces variability and improves consistency for gene expression profiling, especially in analyses of low expressors. Our data indicates that deduplication with MBC shows a higher correlation between observed and expected normalized expression levels (R^2 = 0.93) than no deduplication and deduplication by coordinates alone (R^2 = 0.92 and 0.88 respectively, Figure 5). Furthermore, no deduplication indicates a systematic overestimation of expression levels (Figure 5b) and deduplication with coordinate tends to underestimate the expression level of high expressors (Figure 5c).



A. Following the adaptor ligation step, the SureSelect XT HS2 method provides a parallel workflow for targeted RNA and DNA-Seq library prep. This workflow is compatible with a range of sample input types.

B. The SureSelect XT HS2 mRNA Library Preparation Kit for poly-A selection library preparation.

Figure 1. The modular XT HS2 design enables maximum flexibility.

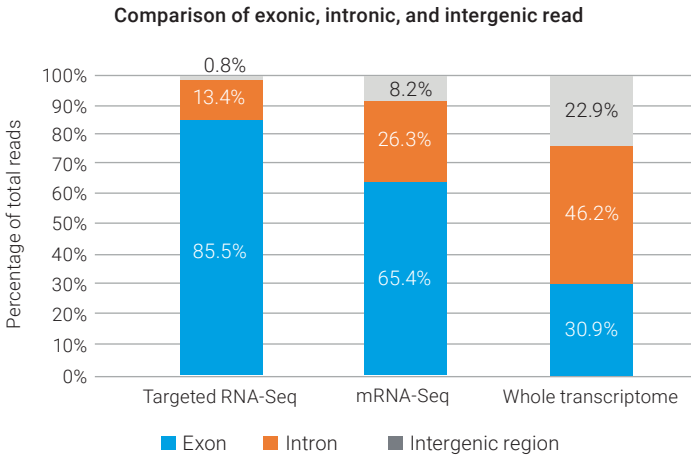


Figure 2. Targeted RNA-Seq provides an effective and economic way to detect gene expression and gene fusions compared to whole transcriptome and mRNA-Seq.

100 ng of total RNA from a lung carcinoma FFPE sample (DV200 = 45%) was used as input. For targeted RNA-Seq, library construction and target enrichment steps were performed using the SureSelect XT HS2 RNA Reagent kit and the SureSelect Human Exon V7 from Agilent. For mRNA-Seq, the library was prepared using the SureSelect XT HS2 mRNA Library Preparation kit. For whole transcriptome sequencing, the library was prepared with RiboMinus (Thermo Fisher Scientific) and the SureSelect XT HS2 RNA library preparation kit. Libraries were sequenced (2 x 150 bp) on the Illumina HiSeq 4000 system and sequencing reads were normalized to 20M for downstream analysis. Targeted RNA-Seq libraries demonstrated a significant increase in the number of on-target exonic reads compared to whole transcriptome and mRNA-Seq libraries.

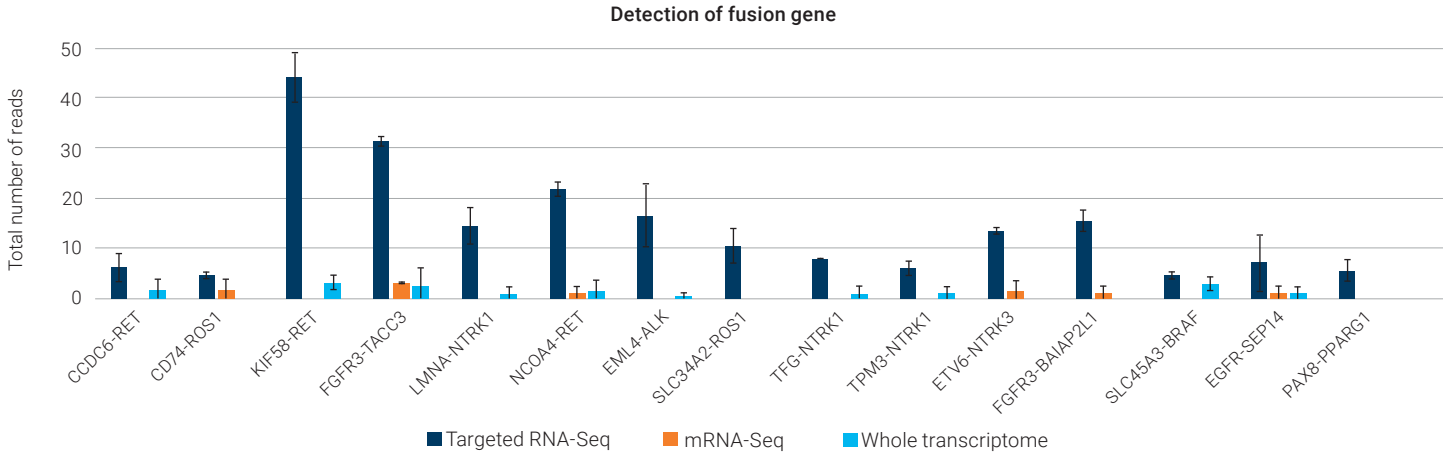


Figure 3. Targeted RNA-Seq offers highly robust fusion detection compared to whole transcriptome and mRNA-Seq methods.

100 ng of total RNA from SeraSeq FFPE Tumor Fusion RNA v4 Reference Material (Material Number 0710-0496, SeraCare) was used as input. All libraries were prepared in two replicates. For targeted RNA-Seq, library construction and target enrichment were performed using the SureSelect XT HS2 RNA Reagent kit and SureSelect Human Exon V7 from Agilent. For mRNA-Seq, libraries were prepared using the SureSelect XT HS2 mRNA Library Preparation kit. For whole transcriptome sequencing, libraries were prepared with RiboMinus (Thermo Fisher Scientific) and the SureSelect XT HS2 RNA library preparation kit. Libraries were sequenced (2 x 150 bp) on the Illumina HiSeq 4000 system and sequencing reads were normalized to 50M for downstream analysis. Targeted RNA-Seq libraries demonstrated a consistently higher number of reads on gene fusions than either whole transcriptome or mRNA-Seq libraries.

Table 1. The overall expression profiles of targeted RNA-Seq libraries are highly correlated to those of whole transcriptome and mRNA-Seq libraries.

		Targeted RNA-Seq		mRNA-Seq		Whole Transcriptome	
		Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
Targeted RNA-Seq	Sample 1						
	Sample 2	0.98					
mRNA-Seq	Sample 1	0.87	0.87				
	Sample 2	0.87	0.87	0.98			
Whole Transcriptome	Sample 1	0.9	0.9	0.91	0.91		
	Sample 2	0.9	0.9	0.91	0.91	0.98	

The table represents R^2 values. 100 ng of total RNA from a fresh-frozen breast tumor sample (DV200=95%) was used as input. All libraries were prepared in two replicates. For targeted RNA-Seq, library construction and target enrichment were performed using the SureSelect XT HS2 RNA Reagent kit and SureSelect Human Exon V7 from Agilent. For mRNA-Seq, libraries were prepared using the SureSelect XT HS2 mRNA Library Preparation Kit. For whole transcriptome sequencing, library preparation was performed with a RiboMinus kit (Thermo Fisher Scientific) and the SureSelect XT HS2 RNA Library Preparation kit. Libraries were sequenced (2 x 150 bp) on the Illumina HiSeq 4000 system. Sequencing reads were normalized to 20M for downstream analysis. Correlation analyses were done based on the regions overlapped by 3 methods. Targeted RNA-Seq libraries demonstrated a high correlation with both whole transcriptome and mRNA-Seq libraries.

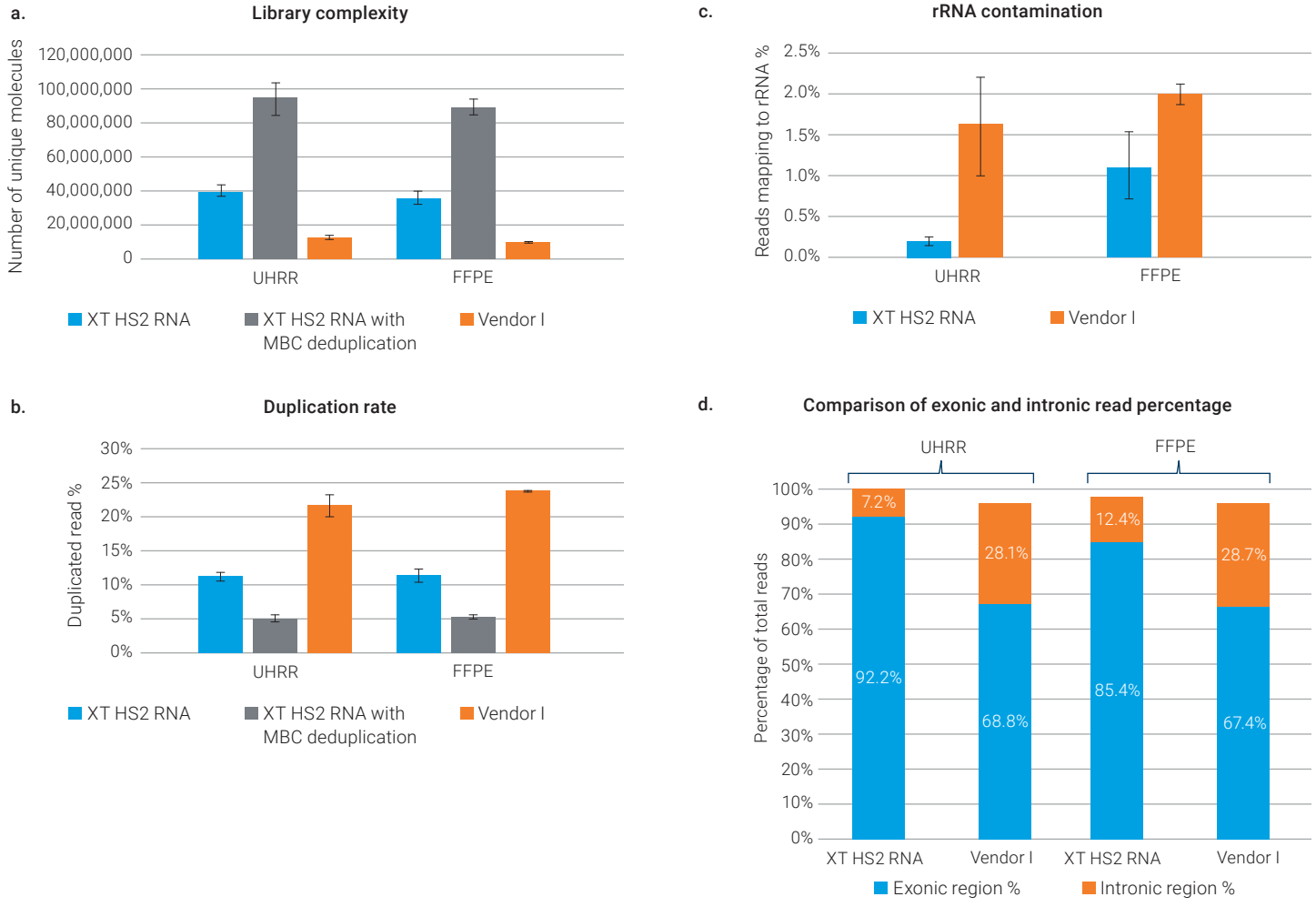


Figure 4. The SureSelect XT HS2 RNA Reagent kit offers superior performance.

100 ng of total RNA from Universal Human Reference RNA (Agilent) (UHRR) or FFPE Tumor Fusion RNA Reference Material v2 (SeraCare) (FFPE) was used as input. All libraries were prepared in two replicates. For XT HS2 RNA, library construction and target enrichment were performed using the SureSelect XT HS2 RNA Reagent kit and the SureSelect Human Exon V7 from Agilent. For Vendor I, library construction and target enrichment was performed using the RNA exome kit from Vendor I. Libraries were sequenced (2 x 150 bp) on the Illumina HiSeq 4000 system. Sequencing reads generated by the SureSelect XT HS2 RNA Reagent kit were analyzed by chromosomal coordinate deduplication without and with MBC information. Sequencing reads generated by Vendor I were deduplicated by chromosomal coordinates only (Vendor I does not offer MBCs). Sequencing reads were normalized to 20M for downstream analysis.

Libraries prepared with the SureSelect XT HS2 RNA Reagent kit showed significantly higher library complexity (panel a) and lower deduplication rates (panel b) across all deduplication conditions compared to Vendor I. The SureSelect XT HS2 RNA Reagent kit also provided a lower rRNA contamination rate and higher on-target (e.g., exonic) coverage than the competitor's kit, demonstrating overall superior performance.

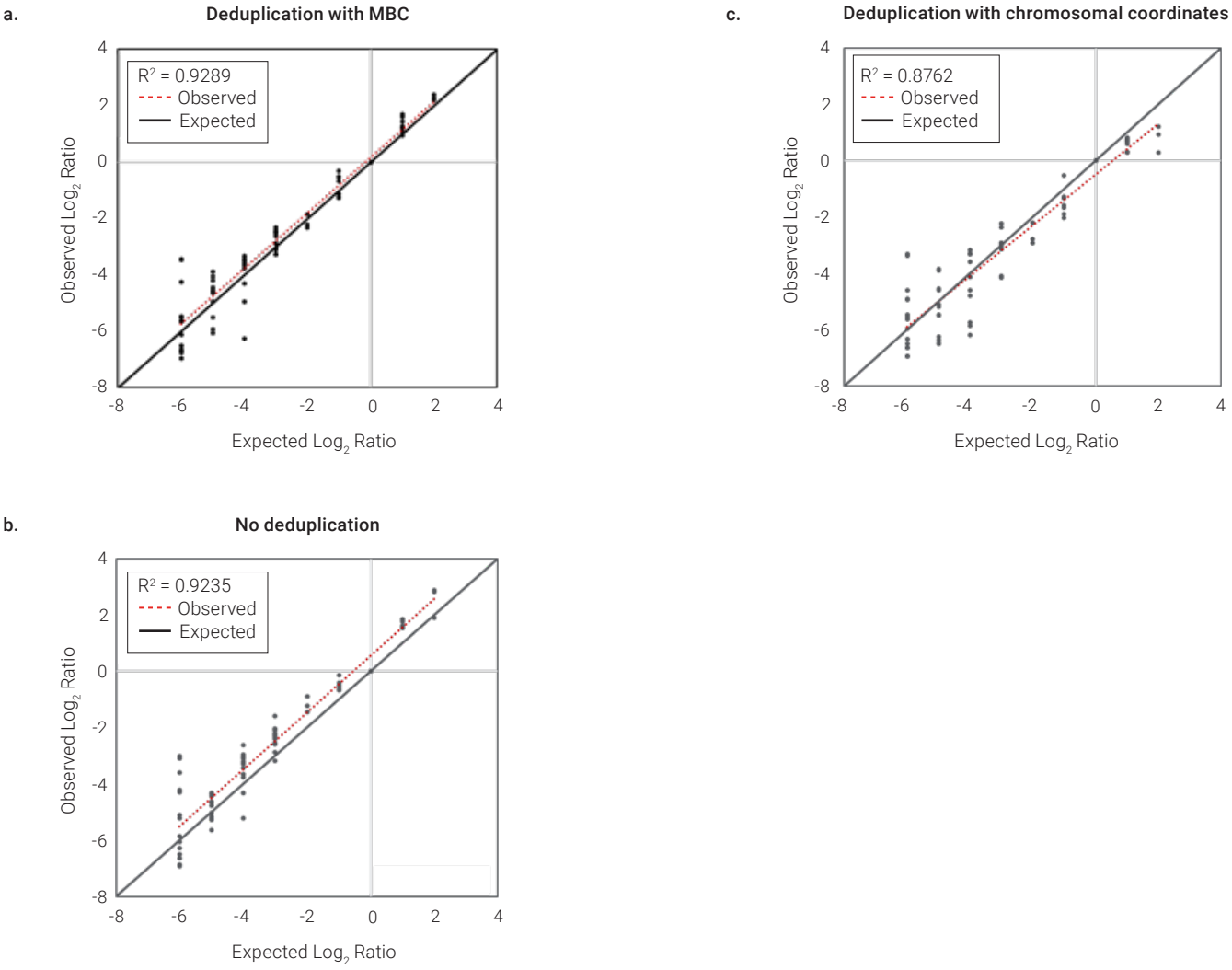


Figure 5. Deduplication with MBC improves gene expression profiling accuracy.

The UHRR sample (Agilent) was spiked-in with a 10,000X dilution of ERCC RNA Spike-In Mix (Thermo Fisher Scientific). 10 ng of total RNA was used as input to generate libraries with the SureSelect XT HS2 RNA Reagent kit and SureSelect Human Exon V7 from Agilent. All libraries were generated in triplicate. Libraries were sequenced (2 x 150 bp) on the Illumina HiSeq 4000 system. 26 ERCC molecules (> 100 attomoles/µL) were selected in each library and used for further analysis. These ERCC expression levels were normalized to ERCC-0004 (transcript ID DQ516752). Sequencing reads were normalized to 1M for downstream analysis.

Deduplication with MBC (panel a) resulted in a higher correlation between observed and expected ratios than either no deduplication (panel b) or deduplication using chromosomal coordinates (panel c), indicating more accurate gene expression data.

Product specification

SureSelect XT HS2 RNA Reagent Kit	
Application	Targeted RNA-Seq
Input	10-200 ng of total RNA
Tissue Sample Type	FFPE, fresh frozen and blood
Strand Specificity	Yes
Turnaround Time	11 hours
Hybridization Time	90-minute fast hybridization
Multiplexity	384 Unique dual indexes (UDI)
Molecular Barcode	Yes
Automation Compatible	Yes (Bravo)

Ordering information

Targeted RNA-Seq	
Product description	**Part number**
SureSelect XT HS2 RNA Reagent Kit with Index Primer Pairs 1-16, 16 Reactions	G9989A
SureSelect XT HS2 RNA Reagent Kit with AMPure® XP/Streptavidin Beads and Index Primer Pairs 1-16, 16 Reactions	G9990A
SureSelect XT HS2 RNA Reagent Kit with Index Primer Pairs 1-96, 96 Reactions	G9991A
SureSelect XT HS2 RNA Reagent Kit with Index Primer Pairs 97-192, 96 Reactions	G9991B
SureSelect XT HS2 RNA Reagent Kit with Index Primer Pairs 193-288, 96 Reactions	G9991C
SureSelect XT HS2 RNA Reagent Kit with Index Primer Pairs 289-384, 96 Reactions	G9991D
SureSelect XT HS2 RNA Reagent Kit with AMPure® XP/Streptavidin Beads and Index Primer Pairs 1-96, 96 Reactions	G9992A
SureSelect XT HS2 RNA Reagent Kit with AMPure® XP/Streptavidin Beads and Index Primer Pairs 97-192, 96 Reactions	G9992B
SureSelect XT HS2 RNA Reagent Kit with AMPure® XP/Streptavidin Beads and Index Primer Pairs 193-288, 96 Reactions	G9992C
SureSelect XT HS2 RNA Reagent Kit with AMPure® XP/Streptavidin Beads and Index Primer Pairs 289-384, 96 Reactions	G9992D
SureSelect XT HS2 RNA Library Preparation Kit with Index Primer Pairs 1-96, 96 Reactions	G9993A
SureSelect XT HS2 RNA Library Preparation Kit with Index Primer Pairs 97-192, 96 Reactions	G9993B
SureSelect XT HS2 RNA Library Preparation Kit with Index Primer Pairs 193-288, 96 Reactions	G9993C
SureSelect XT HS2 RNA Library Preparation Kit with Index Primer Pairs 289-384, 96 Reactions	G9993D
SureSelect XT HS2 RNA Target Enrichment Kit, 12 Hybridizations	G9994A

mRNA-Seq	
Product description	**Part number**
SureSelect XT HS2 mRNA Library Preparation Kit with Index Primer Pairs 1-16, 16 Reactions	G9995A
SureSelect XT HS2 mRNA Library Preparation Kit with AMPure® XP Beads and Index Primer Pairs 1-16, 16 Reactions	G9996A
SureSelect XT HS2 mRNA Library Preparation Kit with Index Primer Pairs 1-96, 96 Reactions	G9997A
SureSelect XT HS2 mRNA Library Preparation Kit with Index Primer Pairs 97-192, 96 Reactions	G9997B
SureSelect XT HS2 mRNA Library Preparation Kit with Index Primer Pairs 193-288, 96 Reactions	G9997C
SureSelect XT HS2 mRNA Library Preparation Kit with Index Primer Pairs 289-384, 96 Reactions	G9997D
SureSelect XT HS2 mRNA Library Preparation Kit with AMPure® XP Beads and Index Primer Pairs 1-96, 96 Reactions	G9998A
SureSelect XT HS2 mRNA Library Preparation Kit with AMPure® XP Beads and Index Primer Pairs 97-192, 96 Reactions	G9998B
SureSelect XT HS2 mRNA Library Preparation Kit with AMPure® XP Beads and Index Primer Pairs 193-288, 96 Reactions	G9998C
SureSelect XT HS2 mRNA Library Preparation Kit with AMPure® XP Beads and Index Primer Pairs 289-384, 96 Reactions	G9998D

www.agilent.com/genomics/XTHS2RNA

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