

# KAPA STRANDED RNA-SEQ *with RiboErase*

Evolved to focus.



The KAPA Stranded RNA-Seq Kit with RiboErase offers a high-quality, comprehensive solution for transcriptome sequencing. By utilizing a targeted enzymatic method for depletion, our workflow enables superior reduction of ribosomal RNA (rRNA) and a more complete representation of the transcriptome, including precursor mRNAs and non-coding RNA (ncRNA). Kits also contain KAPA HiFi for high-efficiency and low-bias library amplification, and include a streamlined, “with-bead” protocol.

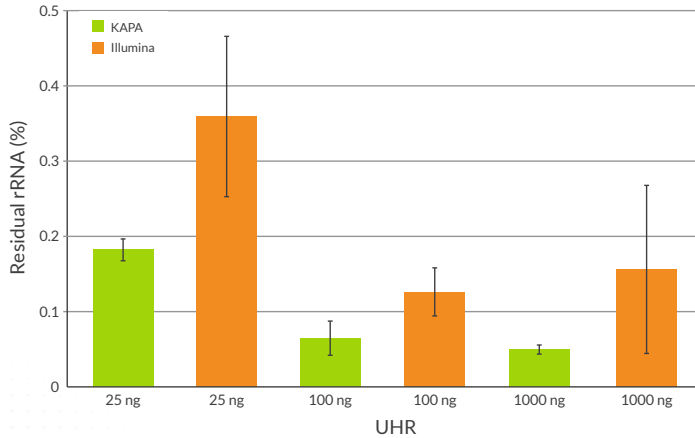
#### Benefits include:

- up to 99.98% rRNA depletion
- flexible input of 100 ng – 1 µg total RNA for human, mouse, or rat species
- robust and reproducible results across various input amounts
- an automation-friendly workflow

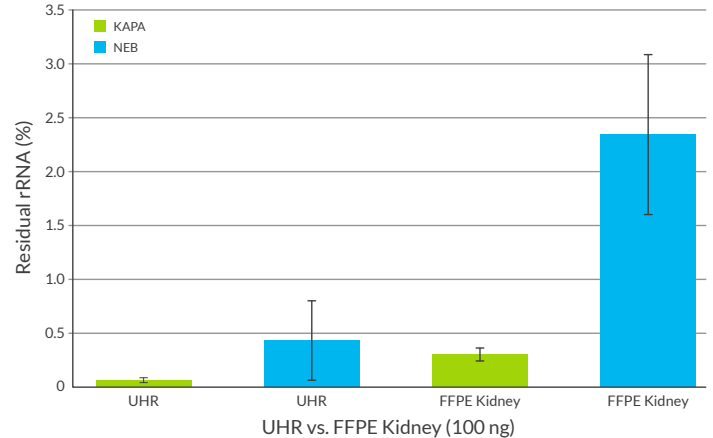
# Industry-leading rRNA Depletion

- Superior rRNA depletion from high-quality and FFPE samples
- More economical NGS sequencing due to decreased rRNA reads, providing deeper sequencing of transcripts of interest

**KAPA RiboErase vs. Illumina Ribo-Zero Gold**



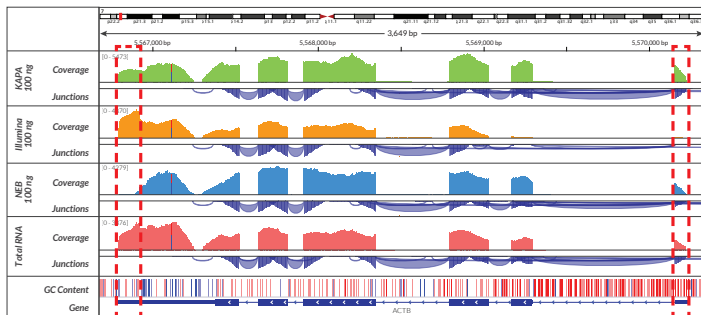
**KAPA RiboErase vs. NEBNext rRNA Depletion**



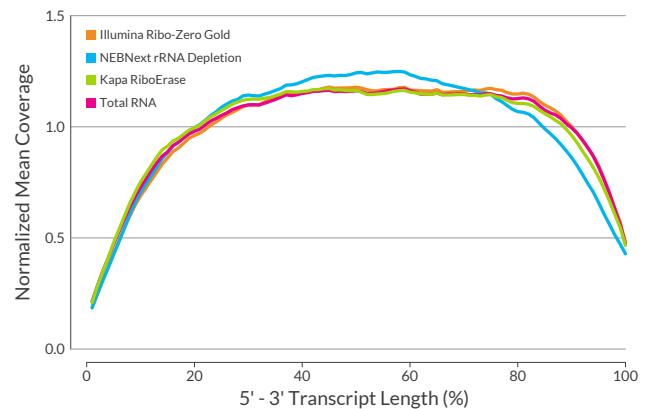
**KAPA RiboErase consistently outperforms Illumina® Ribo-Zero™ Gold and the NEBNext® rRNA Depletion kits across various input amounts and RNA qualities, with only trace quantities of rRNA remaining post-depletion.** In the graph to the left, a wide range of Universal Human Reference (UHR) RNA inputs were depleted in duplicate using both the KAPA Stranded RNA-Seq Kit with RiboErase and Illumina TruSeq® Stranded Total RNA with Ribo-Zero Gold workflows. In the graph to the right, 100 ng of UHR and FFPE RNA (RIN < 3) were depleted using the KAPA Stranded RNA-Seq Kit with RiboErase and NEBNext rRNA Depletion Kit. All samples were sequenced and residual rRNA levels in the samples are represented as a percentage of the total mapped reads.

# Maximum Coverage Uniformity

- Uniform distribution of reads over each transcript
- Minimal 5' - 3' bias across transcripts



**Improved coverage of the whole transcript.** The housekeeping gene Beta-Actin has improved coverage at the 3' and 5' regions using the KAPA Stranded RNA-Seq Kit with RiboErase (green) when compared to the equivalent Illumina Ribo-Zero Gold (orange) and NEBNext (blue) kits.



**Minimal positional bias.** With 100 ng of high-quality RNA input, 3' positional coverage bias is minimized. Compared to Illumina Ribo-Zero Gold and the undepleted sample, KAPA Stranded RNA-Seq Kit with RiboErase produces equivalent 5' - 3' coverage across transcript. When compared to NEBNext, the KAPA Stranded RNA-Seq Kit with RiboErase provides more uniform distribution of reads per transcript.

Unless otherwise stated in references, presented data on file

For Research Use Only. Not for use in diagnostic procedures.

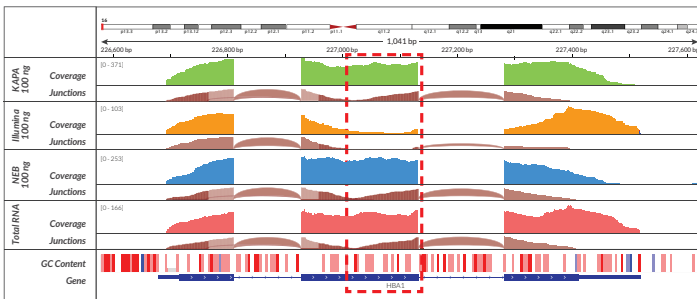
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# High-quality Sequencing Data

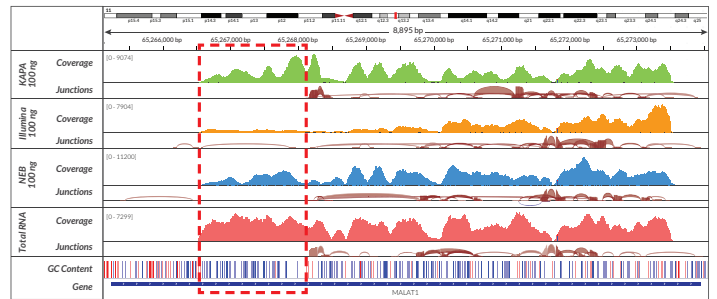
- Detection of more genes and unique transcripts
- Accurate and clear identification of splice sites and alternative gene splicing
- Improved coverage enabling better detection of difficult and GC-rich transcripts

Kit	High-quality sample input	Mapping (%)	Duplication (%)	Coverage uniformity (CV)	Residual rRNA (%)	Strandedness (%)	# Unique transcripts	# Genes identified
KAPA Stranded RNA-Seq Kit with RiboErase	1000 ng	91.585	12.69	0.568	0.049	99.64	24,485,153	22,275
Illumina® TruSeq® Stranded Total RNA Library Prep Kit	1000 ng	92.577	21.77	0.663	0.156	99.95	23,047,496	21,782
KAPA Stranded RNA-Seq Kit with RiboErase	100 ng	90.303	26.77	0.618	0.064	99.5	19,824,805	22,376
Illumina TruSeq Stranded Total RNA Library Prep Kit	100 ng	92.609	34.04	0.639	0.126	99.94	19,425,920	21,898
NEBNext® Ultra Directional RNA Library Prep Kit with NEBNext rRNA Depletion Kit	100 ng	92.393	68.68	0.657	0.432	96.94	8,549,714	22,200
KAPA Stranded RNA-Seq Kit	100 ng (Undepleted)	90.800	11.49	0.684	95.757	99.73	8,148,976	18,361

**High-quality sequencing data obtained from KAPA Stranded RNA-Seq Kit with RiboErase when compared to the equivalent Illumina or NEB kits.** With equivalent mapping rates, KAPA Stranded RNA-Seq Kit with RiboErase provides lower duplication rates, better coverage uniformity and consistently detects more unique transcripts and genes. This enables a more sensitive detection of the expressed genes within the sample.



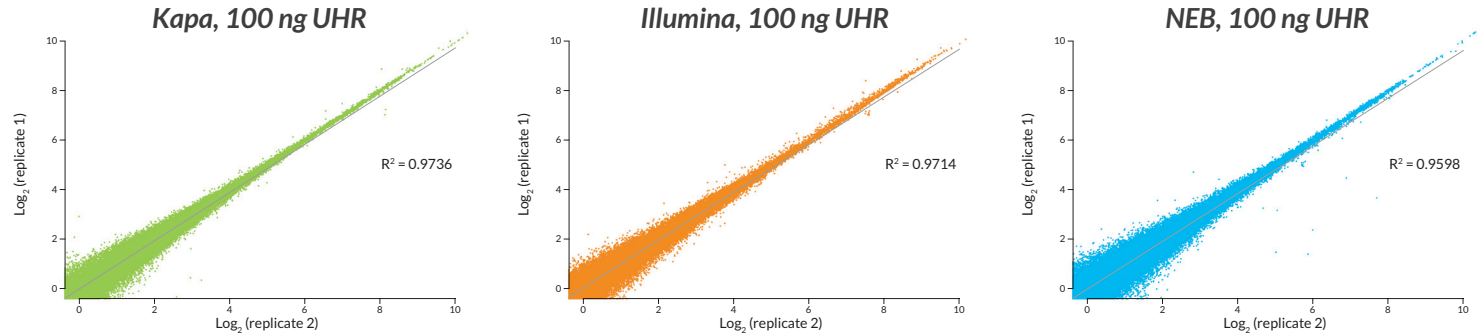
**Improved coverage of GC-rich transcripts.** Coverage and splice junction tracks of the haemoglobin alpha transcript (HBA1), which has a GC content of 62.8%. The GC-content track shows GC-rich regions in red, and AT-rich regions in blue. More even coverage, improved splice-site recognition, and more representative plot compared to the undepleted sample (red) is seen when libraries are prepared with the KAPA Stranded RNA-Seq Kit with RiboErase (green) compared to the equivalent Illumina (orange) or NEB (blue) kits.



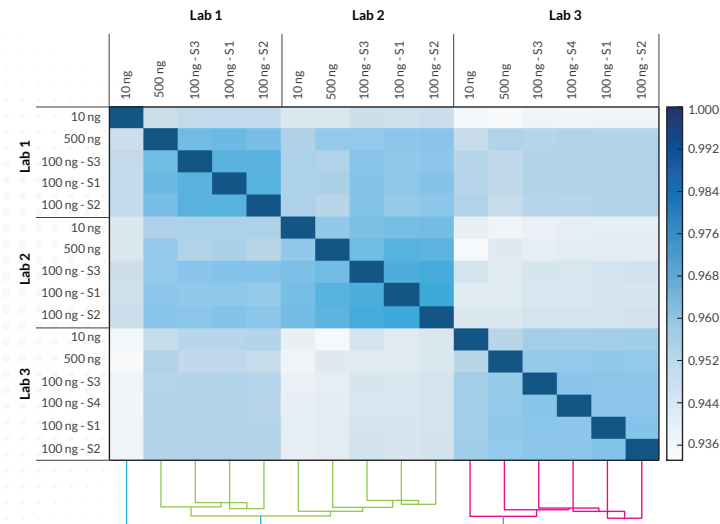
**Better detection of low-abundance transcripts.** Coverage and splice junction tracks of MALAT1, a low-abundance long non-coding RNA transcript, is more comprehensively covered with use of KAPA Stranded RNA-Seq Kit with RiboErase (green) for library preparation. In comparison, Illumina Ribo-Zero Gold (orange) and NEBNext (blue) show less even coverage of this transcript.

# Highly Reproducible Sequencing Results

- High correlation even between different testing conditions
- Low sample-to-sample variation for more reliable results



**Correlation of gene expression levels between replicate samples.** Libraries prepared using 100 ng of UHR Total RNA with the KAPA Stranded RNA-Seq Kit with RiboErase show a very high correlation ( $R^2 > 0.973$ ) which are better than libraries prepared from the equivalent kits from Illumina® or NEB.



**Hierarchically clustered heatmap showing the correlations of gene expression levels between different laboratories and RNA input amounts.** All correlations were greater than 0.93, with technical replicates produced in the same lab exceeding 0.98. Generally, all libraries from a single lab—including those produced from different input amounts—clustered together. The data indicates that the KAPA Stranded RNA-Seq Kit with RiboErase delivers robust, reproducible, and unbiased gene expression data from a wide range of input amounts.

## Ordering Information

Roche Cat. No.	Kapa Code	Description	Kit Size
07962282001	KK8483	KAPA Stranded RNA-Seq Kit with RiboErase HMR	24 reactions
07962304001	KK8484	KAPA Stranded RNA-Seq Kit with RiboErase HMR	96 reactions

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