RNA Input Recommendations

The Ribo-Zero Plus protocol is optimized for 10–100 ng RNA input. The protocol accepts 1–1000 ng purified total RNA input from high-quality RNA samples (RIN ≥ 9) and accepts 10–100 ng RNA input from low-quality RNA (RIN ≥ 2) samples or FFPE (DV200 > 55%) samples. Library performance can vary with lower input amounts and lesser quality RNA.

The Ribo-Zero Plus Microbiome protocol is optimized for 25-500ng total RNA input. RNA is recommended to be at least RIN ≥ 5. Library performance can vary with lower input amounts and lesser quality RNA.

The protocols can deplete rRNA from the following targets.

The Illumina Total RNA with Ligation Ribo-Zero Plus Microbiome (96 reactions)(Cat#20072063) depletes microbiome rRNA. Refer to the items listed in the table for more details.

Depletion Target	rRNA Target
Human cytoplasmic rRNA	28S, 18S, 5.8S, and 5S,
Human mitochondrial rRNA	12S, and 16S
Human beta globin transcripts	HBA1, HBA2, HBB, HBG1, and HBG2
Mouse and rat	12S, 16S, 28S, 18S, 5.8S, and 5S
Gram(-) bacterial rRNAs	*E.coli*: 5S, 16S, 23S
Gram(+) bacterial rRNAs	*B. subtilis*: 5S, 16S, 23S
Microbiome rRNA*	5S, 16S, 23S from diverse microbes including common adult, infant bacterial species and ATCC MSA-2002, MSA-2005, and MSA-2006

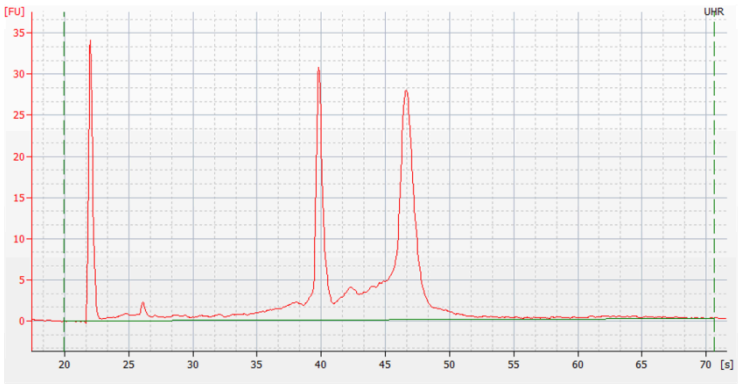
* Only included in Illumina Stranded Total Ribo-Zero Plus Microbiome kit (Cat#20072063). The rRNA depletion was optimized for human microbiome rRNA, performance can vary when using other sample types.

Include a DNase treatment with the RNA isolation method. DNase treatment must be done while running your preferred extraction method. The DNase treatment ensures sample purity and accurate quantification. Before starting the protocol, quantify the total RNA with a fluorometric method intended specifically for RNA. Assess quality using a fragment analysis method.

The following figures provide example traces for Universal Human Reference (UHR) total RNA input.

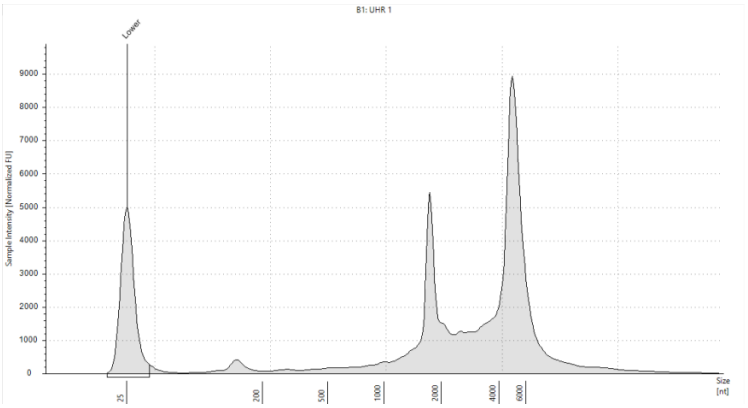
Example Bioanalyzer Trace

Figure 1: 2100 Bioanalyzer System trace using RNA 6000 Pico Kit



Example TapeStation Trace

Figure 2: 4200 TapeStation System trace using RNA ScreenTape



Check Library

This step checks the concentration and quality of the final libraries.

1. Analyze the library using one of the following methods:
   - If using the NextSeq 1000/2000 Sequencing System, dilute 1 µl library 1:10,000 dilution, and then analyze library using KAPA qPCR library quantification kit.
   - For all other sequencing systems, analyze 1 µl library using one of the following:
     - Agilent 2100 Bioanalyzer and DNA 1000 Kit
     - TapeStation System and D1000 ScreenTape

   The following figures provide example traces of final libraries generated from 10 ng and 100 ng Universal Human Reference (UHR) RNA input.

Example Bioanalyzer Traces

Figure 1: Bioanalyzer trace with 10 ng input

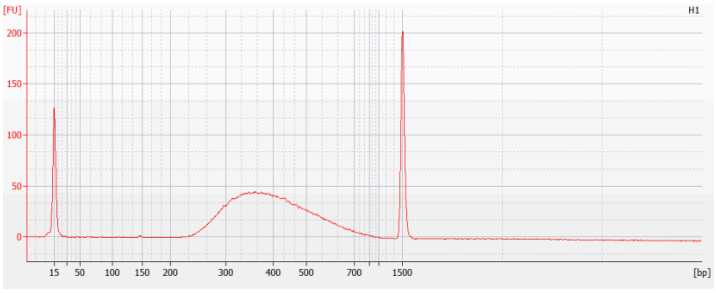


Figure 2: Bioanalyzer trace with 100 ng input