

# What is RNA Integrity?

RNA is inherently unstable and degrades easily. This makes RNA challenging to deliver in a therapeutic before it degrades or is rejected by the cell. However, RNA therapeutics and vaccines protect human health and treat diseases, so confidently measuring RNA integrity is important to continue advancing RNA medicines.

RNA integrity is a measure of how intact and stable a strand of RNA is. High integrity RNA samples consist of primarily full-length molecules and very few fragmented molecules. RNA with high integrity performs more effectively as therapeutics since only full-length molecules produce therapeutic proteins and fragmented molecules tend to elicit unwanted immune responses.

There are two categories of RNA integrity analysis: Total RNA integrity and IVT RNA integrity.

## Integrity of Total RNA

Total RNA is a sample of RNA extracted from cells and tissues that contains all elements and types of RNA. Drug developers typically determine Total RNA integrity by calculating the RNA Integrity Number (RIN). RIN is a measure of the quality of an RNA sample to be used in a transcriptomic experiment. Its value ranges from 1-10, with 1 being severely degraded RNA and 10 being intact, high-quality RNA. RIN was developed by Agilent Technologies.

To measure RIN, drug developers prepare their Total RNA in an RNA Nano chip to use in an Agilent Bioanalyzer. The Bioanalyzer analyses the chip's RNA sequence, noting the 18S and 28S peaks, for the RNA's stability, integrity, and performance across the transcriptome. Once analyzed, the algorithm provides the RIN for the sample.

A similar analysis measures the RINe (RNA integrity number equivalent) with an Agilent TapeStation. For this analysis, drug developers prepare their Total RNA sample, buffer, ladder, and tape to input in the TapeStation. Once inserted, the machine performs electrophoresis and analysis itself, studying the 18S and 28S peaks and providing a RINe. While RINe works very similarly to RIN, the algorithms and metrics are not identical.

The TapeStation can provide numerous samples quickly, running up to 96 Total RNA samples at once compared to the Bioanalyzer's 12 samples. However, the Bioanalyzer method is established as the "gold standard" for Total RNA integrity, making it a recognizable and reliable method.

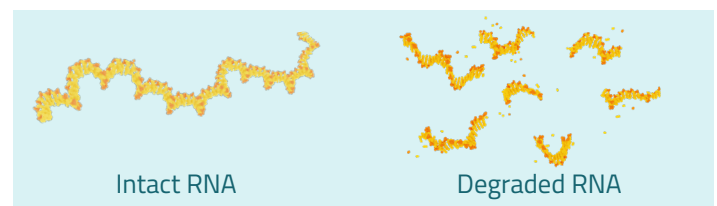
## Integrity of IVT RNA

IVT (in vitro transcribed) RNA is a synthetic RNA of a single RNA species. In the therapeutics space, IVT RNA is often an mRNA sequence. Drug developers cannot calculate the RIN of IVT RNA since it is a single species of RNA. Rather, they determine what percentage of a sample is intact compared to what is expected.

Drug developers can measure IVT RNA integrity using the TapeStation. The preparation methods are the same as using the machine for Total RNA, but instead of calculating RIN, the algorithm determines the size of the sample compared to the expected size. The closer the size and number of nucleotides in the sample is to the expected size, the more intact the IVT RNA is.

Another method to measure IVT RNA integrity is using Agilent's Fragment Analyzer. In this method, drug developers perform capillary electrophoresis, and the analyzer measures the size or length of the RNA. This method has a higher resolution than the TapeStation, offering more detailed insights into specific nucleotide-level sizes. However, the TapeStation is more flexible for various sample sizes, quickly running anywhere between 1-96 samples at once.

Beyond the methods listed above, sequencing is a newer method to measure IVT RNA integrity. Nanopore and next-generation sequencing can offer insights into IVT RNA integrity at a single nucleotide level by measuring the IVT RNA sequence, highlighting specific fragments and regions prone to breakage.



## RNA Integrity at Eclipsebio

At Eclipsebio, we prioritize the need for high integrity RNA for therapeutics. We use [nanopore sequencing](#) to directly measure IVT RNA, locating potential places of degradation at a single nucleotide level. Our new [eSENSE Break](#) assay uses next-generation sequencing to map breakage hotspots and reveal why fragmentation occurs, providing insights on how to improve integrity for an effective therapeutic.

Interested in how Eclipsebio can reveal insights into your RNA's integrity? [Contact us](#) to get started.