

# Testing Protocol

## Why Rainin Does Not Test for RNA

**It may appear curious to some customers that Rainin tests its tips for human DNA contamination, but conducts no such tests for contaminating RNA. The answer, which is provided in some detail below, really has to do with the amount of RNA that is likely to be present, our ability to detect negligible traces of RNA and finally, the necessity to test for RNA, given that we already scrupulously test for DNA.**

First, the sensitivity of Rainin's test for contaminating human DNA informs us with a high degree of certainty that there cannot possibly be contaminating RNA present, beyond the faintest possible trace. Hence, a separate test for RNA is neither required nor possible, based upon the sensitivity of various test methods, as explained below.

A typical diploid human cell with two copies of DNA contains approx. 7 pg of genomic DNA (gDNA) and approximately 0.1-1 pg (avg. = 0.5 pg) of total cellular RNA. The RNA is divided into three main types or families: 1. Messenger RNA (mRNA), 2. Ribosomal RNA (rRNA) and 3. Transfer RNA (tRNA). In terms of predominant mass, rRNA and tRNA make up the bulk of RNA within the cell. These are structural and functional RNA types and do not code for proteins. The mRNA pool consists of a spectrum of mRNA species, each coding for a different protein. Some mRNAs are fairly abundant and others are represented by only a few copies.

Rainin's human DNA test procedure has an LOD of 3.2 pg. This means that our test will detect down to about a single copy of human gDNA. This would be equivalent to a single contaminating haploid cell. At that level of detection, the accompanying RNA would be expected to have an average mass of approx. 0.05-0.5 pg (average = 0.0275 pg). Also, the half-life of mRNA in a "viable" cell is <24 hours. This means that a contaminating human cell (the source for any DNA and RNA contamination on a Rainin tip) would almost certainly contain very little (if any) RNA at the time of Quality testing. This is largely because RNA is rather quickly degraded if the cell is traumatized or the RNA is exposed to the environment. For the sake of discussion, let's

say that the accompanying RNA was abundant, with a mass of 0.5 pg. If completely intact, all anticipated contaminating human RNA would be less than one sixth ( $3.2/0.5 = 6.4$ ) the mass amount of DNA in a haploid cell and less than  $1/12$ th the mass amount of DNA in a typical human diploid cell. This means that there is no plausible scenario in which the mass of contaminating RNA would substantially exceed (or could exceed at all) the amount of contaminating DNA. Since the DNA and RNA are always present together in the cell, the amount of contaminating DNA will always exceed the amount of contaminating RNA accompanying it. RT-qPCR would be the most sensitive method that we could invoke to test for any contaminating RNA and for most RT-qPCRs, you would need at least 5 ng of total RNA to be measurable. You can see that even if we had 0.5-1 pg of total RNA accompanying the 3.2 pg of gDNA. We would only have  $1/10,000$ th to  $1/5,000$ th the amount (at best) of RNA required to perform an RT-qPCR.

Since the DNA and RNA are together in the cell, they must be together as contaminants on our tips as well. If we can't detect 3.2 pg of human gDNA, this tells us that any accompanying RNA is not only non-detectable and can only be present in an amount which is so vanishingly small (if present and intact at all) that it could not possibly interfere with even the most sensitive customer assay, which is RT-qPCR. Hence, we do not believe that a separate RNA test is required or would be likely to succeed, if attempted.

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Local contact: [www.mt.com/contacts](http://www.mt.com/contacts)

Subject to technical changes

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