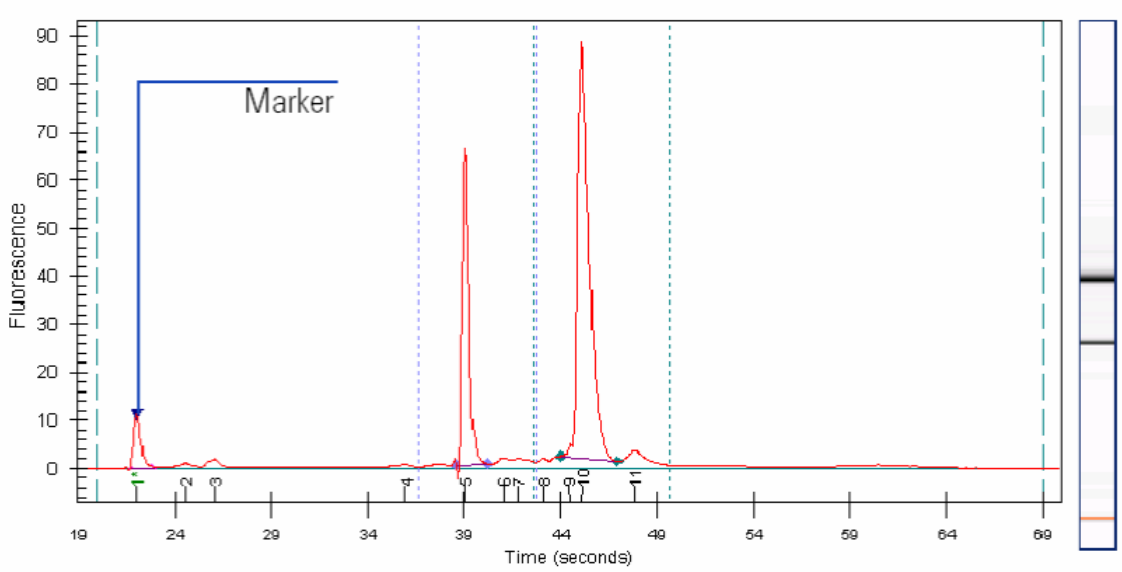
Total RNA Sample Results



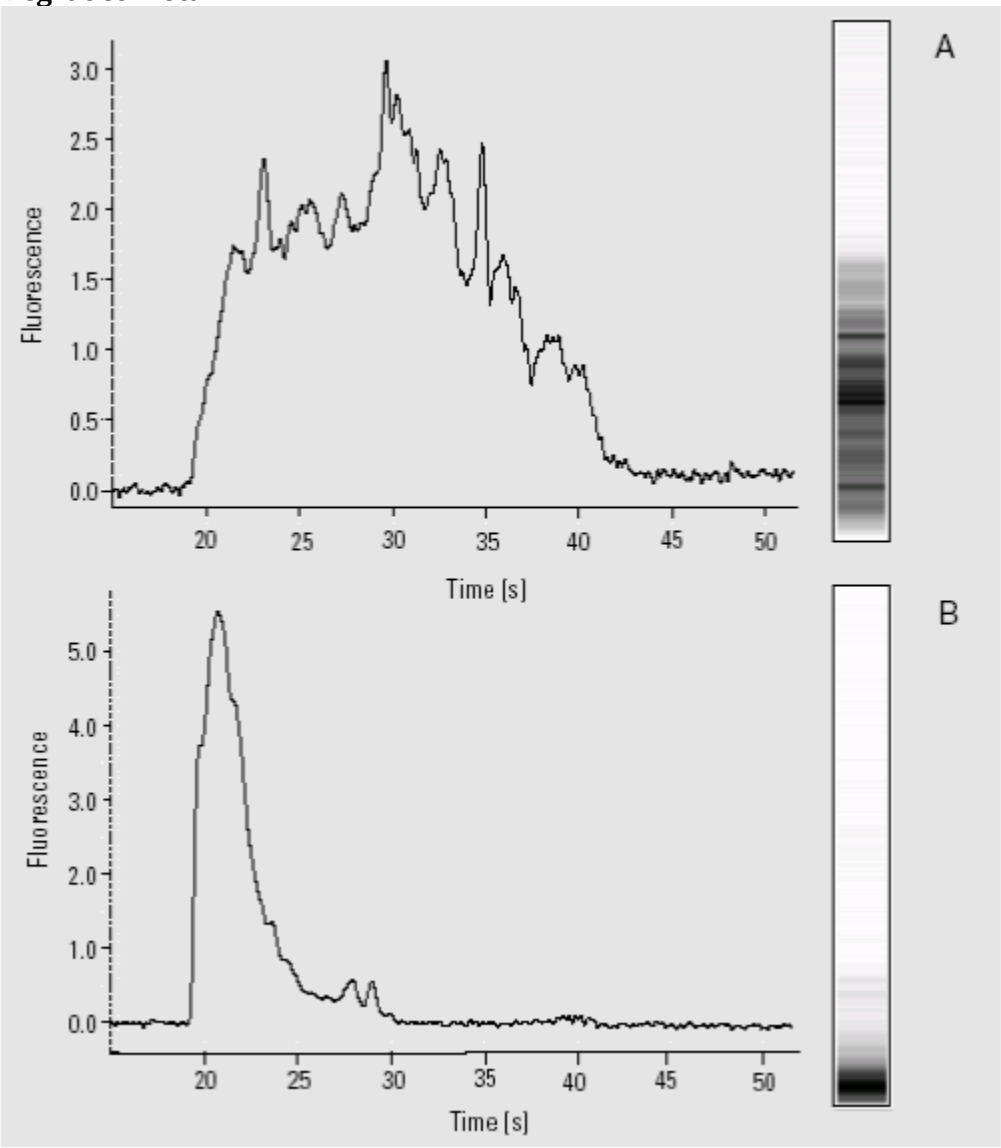
The Bioanalyzer electropherogram of total RNA shows two distinct ribosomal peaks corresponding to either18S and 28S for eukaryotic RNA or 16S and 23S for prokaryotic RNA and a relatively flat baseline between the 5S and 18S ribosomal peaks.
Major features for a successful total RNA run are:

- Two ribosomal peaks: Any mRNA migrating between the ribosomes will be smooth and lack distinct peaks.
- The baseline between 29 seconds and the 18S ribosomes is relatively flat and free of small rounded peaks corresponding to smaller RNA molecule. Depending on the RNA extraction method, the small 5S, 5.8S and tRNA may be present in the electropherogram from 24-27 seconds.
- 1 marker peak

Ribosomal RNA ratio and baseline for the quality assessment of RNA sample

In general, the height of the 28S ribosomal peak should be twice that, or at least equal to the 18S ribosomal peak. Variability in this ratio may indicate partial degradation of the sample by ribonuclease contamination during the purification procedure. In the case of complete sample degradation these bands will disappear. However, ribosomal ratios will vary according to the species and tissue type as well according to the RNA extraction method. For a specific tissue, similar values should be found so that after establishing quality criteria for a specific sample type, the ribosomal ratio can be used as a quick check for RNA sample quality.
Beside the ratio, the baseline is a kind of trademark and could be used as a secondary parameter for the RNA quality. If you have a flat baseline, your RNA is OK. The more peaks visible (and higher background) between the two ribosomal bands and below the 18S (16S) band, the worse the sample quality. In the lower part of the electropherogram around 24 to 29 seconds, 5S RNA and transfer RNA can be seen because they cannot be separated on the Bioanalyzer. Typically, trizol isolations do not remove 5S and tRNA, while many column-based RNA extraction kits do remove these small RNA species. In Eukaryotic cells the 5S/tRNA should represent 5 to 10 % (maximum) of the 18S and the 28S. This population of small fragments is composed of several populations from 5S to 8S and the profile is tissue- and cell-specific. In some Prokaryote cells, there is 20% of messenger and 30 to 40 % of 5S/tRNA (information from Agilent Technology Inc)

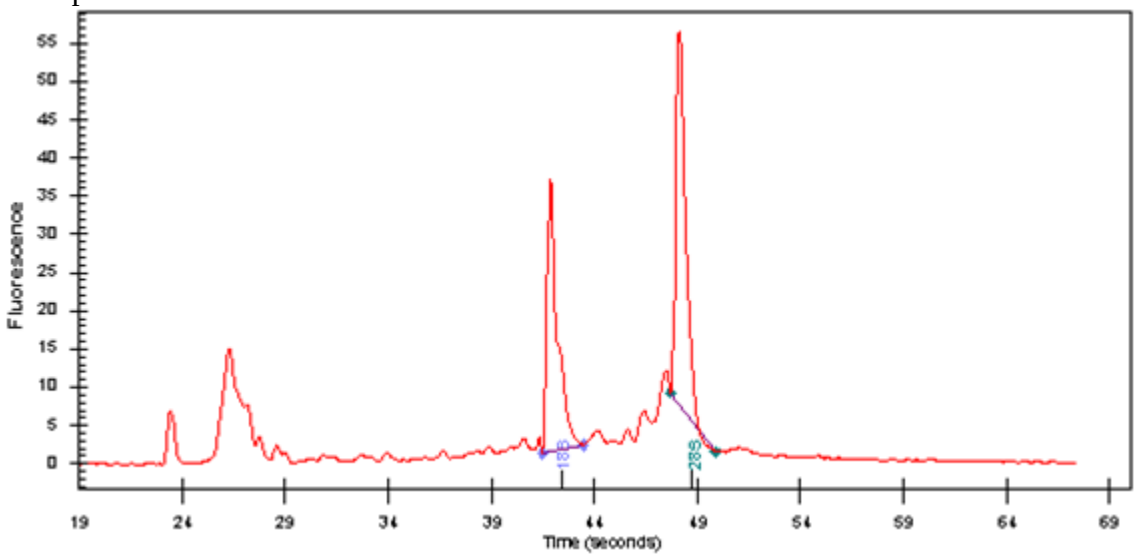
Degraded Total RNA



RNase degradation of total RNA samples produces a shift in the RNA size distribution toward smaller fragments and a decrease in fluorescence signal and the 18S and 28S peak can no longer be identified with certainty (figure A). Degraded total RNA will lack a smooth baseline and typically contains multiple peaks that are as large or larger than the ribosomal peaks. The most highly degraded products have a migration time between 22 and 24 seconds. With more severe degradation (figure B), the spectrum shifts entirely toward early migration times.

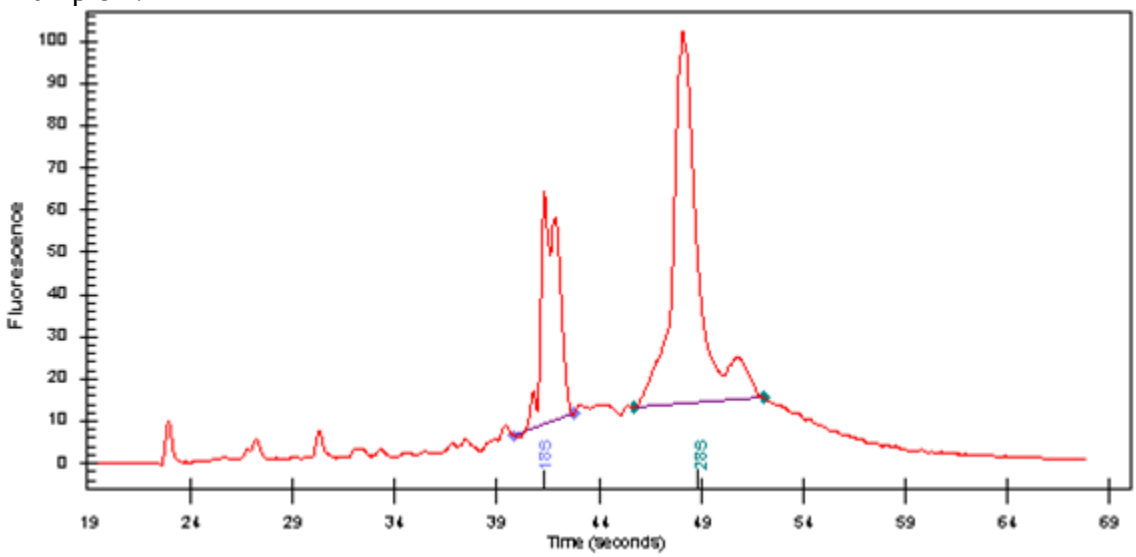
Contamination in Total RNA

Example 1:



The peak seen here migrating between 24 and 29 seconds is most likely tRNA, 5S ribosomal RNA, or a combination of both.

Example 2:



The bump running just after the 28S ribosomal peak is genomic DNA contamination. It is suggested a DNase1/RNase free treatment to get rid of this genomic DNA contamination.