

Ask Frank

by Fred and Hank



FRED HYDE



HANK DAUM

Questions about RNA Extraction from formalin-fixed, paraffin-embedded (FFPE) tissues

Q. Why is it so difficult to isolate good quality RNA from FFPE tissues?

A. The process of fixing the tissue sample and embedding it in paraffin can cause severe degradation of the RNA. In general, RNA isolated from more recently embedded tissues will be of better quality than RNA isolated from older embedded samples. The formalin fixation process causes cross-linkage between nucleic acids and proteins, and the covalent modification of RNA by mono-methylol (-CH₂OH) addition to the bases.¹ This cross-linking inhibits reverse transcription of the extracted RNA, and makes it difficult to do cDNA synthesis. Thus, in order to isolate the best quality RNA from FFPE tissues, a dependable method is required for extraction of RNA from the cross-linked matrix.

Q. When I isolate RNA from FFPE tissues, what is the quality as compared to RNA isolated from fresh tissues?

A. The quality of the RNA from paraffin-embedded tissues will be much lower. The RNA isolated from FFPE tissues will be shorter (primarily as fragments of less than 300 bases in length), and the yields will be lower than those obtained from the same mass of fresh tissue. In addition, when amplified with oligo-dT primers and used in microarrays, there will be a much higher 3'-bias in the data generated due to the fragmented nature of the RNA following extraction from FFPE tissues. Given this situation, one should not directly compare results between intact and degraded RNA. Analysis of RNA isolated from FFPE tissues by electrophoresis will often show an almost complete disappearance of the intact

ribosomal RNA bands; however, this may not mean that the messenger RNA is completely degraded.

Q. Can I isolate RNA from FFPE tissues using the ArrayPure™ Nano-scale RNA Purification Kit or MasterPure™ RNA Purification Kits?

A. Yes. There are a number of recent publications that cite the use of EPICENTRE Biotechnologies' MasterPure RNA Purification Kit in the isolation of RNA from FFPE tissues. For example, in the paper by Cronin *et al.*,² the authors cite using the MasterPure Purification Kit, as per the manufacturer's recommendations, to isolate RNA from 10 micron slices of archival breast tumor tissue section blocks.

Q. Can I use the RNA isolated from FFPE tissues with the ArrayPure or MasterPure RNA Kits for quantitative RT-PCR (qRT-PCR)?

A. Yes, you can.

Q. If I am using TargetAmp™ aRNA Amplification Kits or the MessageBOOSTER™ cDNA Synthesis Kit to amplify RNA from FFPE tissues for microarray or qRT-PCR analysis, what kind of results can I expect?

A. In general, one will see highly elevated 3'/5' ratios due to the degradation of the RNA, and when the amplified RNA is analyzed on an Agilent 2100 bioanalyzer the amplified RNA will be shorter than amplified RNA from fresh samples. This does not necessarily mean

that the results are suspect, our customers have found that RNA isolated from FFPE tissues is a good amplification substrate. The amplified RNA generated from RNA isolated from FFPE tissues can still provide highly useful expression data and can also be used successfully in qRT-PCR.

Q. How does the age of the Paraffin block affect the quality of the RNA obtained with the MasterPure RNA Purification Kit?

A. The age of the FFPE tissue blocks will of course have an effect on the quality of the RNA isolated. It is estimated that after 10 or more years, the quality of the RNA degrades significantly. However, recent customer studies indicate that RNA isolated from 25-year-old FFPE tissues was still a usable substrate for amplification by the TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit (unpublished data, personal communication), and the resulting amplified RNA was suitable for use in microarray studies.

References

1. Masuda, N. *et al.*, (1999) *Nucleic Acids Res.* **27**(22), 4436.
2. Cronin, M. *et al.*, (2004) *Am. J. Pathol.* **164**(1), 35.

