DOI:

Ke d : RNA; RNAlater; TRIzol; RNase; Allprotect; PAXgene

I d c

Gene expression analysis provides information at which a particular gene pattern may be expressed by cellular responses. Gene expression is evaluated by means of reverse transcription polymerase chain reaction (RT-PCR), quantitative real-time RT-PCR (qRT-PCR) as well as multiplex gene expression quantification arrays and, data analysis depends on the RNA integrity and stability isolated from cells [1]. RNA quality is influenced by warm and cold ischemic durations, cellular stress responses, tissue processing protocols and storage conditions [2,3]. Isolation of highly pure intact RNA is vital for successful quantification of gene expression [1,4] so that RNA preservation is an essential subject during handling processes [5]. Since RNA degradation takes place by cytoplasmic RNase [4] thus, RNase inhibition is a main approach in RNA extraction and subsequently gene expression analysis [1,4].

C e e d

F a a

RNA degradation correlates with the length of delay in sample fixation or preservation. Most pathology departments still rely on tissue preservation with formalin fixation followed by paraffin embedding step [3]. Although it is suitable for morphologic assessment, it is unable to keep intact RNA [5].

In fact, formalin fixative keeps tissue structure via cross-linking of cellular proteins. In addition, there also happen cross-links between proteins and nucleic acids. Mono-methylol groups (-CH_2OH) added to all four bases of RNA create methylene bridges between two amino groups. Although it makes formalin an ideal fixative hardening agent for histopathological analysis, cross-linking of proteins and nucleic acids causes intact mRNA extraction from formalin-fixed tissues to be difficult [3,4,6,7]. RNA extracted from formalin-fixed paraffin-embedded (FFPE) tissues is chemically modified and dramatically degraded. It is often present in fragments less than 300 bases in length [4,5,8,9] and may produce reliable data if selected genomic sections be heavily amplified using specific primers and short amplicons (<300 bps) [4,10]. In addition, RNA chemical modifications in FFPE tissues can be reversed with proteinase K digestion and preheating in guanidinium or TE (10 mM Tris-HCl, pH 7.0, 1 mM EDTA) or citrate (pH 4.0) buffers making it more amenable to RT-PCR yet, the major drawback of formalin fixation (i.e. fragmentation) remains uncorrected [4,9,10].

85(l5uta6)7yCuta75(lCuthndk119auta)3(s9uthndk119aut8)7(y85(autyCuts63(su)45Futhndk119aut86uts63(sC7(y85(l1)7s63(s8uta6)7hFuthndk101aut87

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RNA a e e e e a

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