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COMPARISON OF COLUMN-BASED KIT AND MANUAL METHOD FOR RNA EXTRACTION FROM ANIMAL TISSUE

Mohsen Foroughi Abari[1*], Shahin Eghbalsaied[1], Stefan Wagner[2], Kamran Ghaedi[3]
[1]Department of Animal Science, Isfahan (Khorasgan) branch, Islamic Azad University, Iran;
[2]AgResearch, Ruakura Research Centre, Hamilton, New Zealand; [3]Deaprtment of Biology, University of Isfahan, Iran
[*]corresponding author email address: mohsen.forughi@gmail.com

Objectives: Since the preparation of purified and high-quality RNA is one of the most essential elements in the preparation of cDNA, low-quality extracted RNA can result in improper subsequent analysis. Due to the differences in structure of DNA and RNA, RNA molecule is more sensitive and can be easily decomposed. On the other hands, RNA degradation enzyme, i.e. RNase is abundantly available and broad range and can often act without the need of co-factor. Thus, all the stages through RNA extraction procedure must be controlled and RNase-free devices should be utilized. Given the major role of quantity and quality of extracted RNA on downstream analysis, the present study aimed to compare manual and column-based method for RNA extraction.

Methods: Sheep's heart was used as the RNA origin tissue. Samples were homogenized in nitrogen flask and the RNA extraction was carried out using both manual (RNX plus solution) and column-based method (Hybrid-R, GeneAll, South Korea). In order to scrutiny of the RNA quality, the ratio absorbance of 260:280 (nm:nm) was measured. Impurities such as proteins, phenol and other soluble components in the sample solution which mimic the absorbance ratio, agarose gel electrophoresis was carried out. In addition, the RNAs were treated with DNase enzyme to make sure the DNA-free extraction. To ensure for absence of genomic DNA, PCR was performed on RNA samples. Thereafter, the obtained RNA by the both methods underwent cDNA synthesis using Oligo dT primers followed by PCR by β-Actin primers.

Results: The results showed that using Hybrid-R kit resulted in better RNA extraction in both qualitatively and quantitatively. However, after PCR-reaction, similar results were observed for the both extraction methods. This may be due to this fact that end-point PCR machine can amplify different quantity of amplicons because of large number of cycles, 35 cycles in this experiment. Nevertheless, extraction by the column-based method was more reliable without further requirement of DNase treatment.
Keywords: RNA extraction, sheep tissues, β-Actin, RT-PCR

References

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