Biology | Molecular Biology

RNA Extraction

General Aim

To extract cellular RNA using Trizol reagent method.

Method

Trizol reagent method.

Learning Objectives (ILOs)

- Demonstrate proficiency with the protocol involved in RNA extraction.
- Identify the role of specific reagents and equipment in the extraction of RNA.
- Practice basic laboratory techniques.
- Conclude downstream applications of RNA extraction.

Theoretical Background/Context

- The 'central dogma' of molecular biology illustrates the flow of genetic information from DNA, through RNAs, to proteins. There are several types of RNA in the cell. The messenger RNA (mRNA) is formed during the process of transcription from DNA, which basically means the production of a translatable copy of DNA, eventually leading to the synthesis of a specific protein. mRNA is then involved in the process of translation along with transfere RNA (tRNA) and ribosomal RNA (rRNA). tRNA is the amino acid carrier, it reads the code of the mRNA and adds the appropriate amino acid accordingly.

Theoretical Background/Context (continued)

- rRNA is the factory where the process of translation occurs. Other RNAs include small and long non coding RNAs which are believed to have regulatory impact over gene expression. RNAs were also found to act as enzymes e.g. ribozymes. They sometimes carry the genetic material instead of DNA e.g. viral RNA. Studying RNA thus can be conducted for various reasons; for example, to study gene expression and its regulation. Detection of viral RNA can be used to diagnose infection.

Principle of Work

- Total RNA is isolated and separated from DNA and protein after extraction with a solution called Trizol reagent. Trizol is an acidic solution containing guanidinium thiocyanate (GITC), phenol and chloroform. GITC irreversibly denatures the DNA which is a process in which proteins or nucleic acids lose their quaternary, tertiary and secondary structure, which are present in their native states.
- This is followed by centrifugation. Under acidic conditions, total RNA remains in the upper aqueous phase, while most of DNA and proteins remain either in the interphase or in the lower organic phase. Total RNA is then recovered by precipitation with isopropanol. Finally, RNA washing and purification is done using 70 % alcohol.