
RNA
Extraction

RNA

Ribonucleic acid (RNA) is one of the three major biological macromolecules that are essential for all known forms of life (along with DNA and proteins).

Obtaining **high-quality RNA** is the most critical, step in performing many molecular techniques such as:

- RT-qPCR
- Digital PCR
- Transcriptome analysis using Next-Generation Sequencing
- Northern Blotting
- cDNA library construction

RNA isolation procedures must include some important steps **Before**, **During**, and **After** the actual RNA purification.

- **Treatment and handling of samples prior to RNA isolation**
- **Choice of technologies used to prepare the RNA**
- **Storage of the prepared RNA sample**

Precautions for preventing RNase contamination

➢ Precautions for preventing RNase contamination

- RNase activity is difficult to inhibit, so it is very essential to prevent its introduction.
- Always wear disposable gloves.
- Usually our skin contains many bacteria and molds that can contaminate the RNA preparation and be a source of RNases
- Use sterile, disposable plastic ware.
- Use automatic pipettes reserved for RNA work.

RNA Extraction

➢ Sample Collection and Protection

- For late processing; Freeze the tissue/cells in liquid nitrogen or on dry ice.

- RNA stabilization solutions (e.g. RNA*later*)

- Stored tissues in RNA*later* will be then homogenized in the lysis buffer specified by the selected RNA isolation method.

RNA Extraction

➢ RNA Preparation

- **Organic Extraction Methods**

the sample is homogenized in a phenol-containing solution, centrifuged, the upper aqueous phase is recovered and RNA is collected by alcohol precipitation and rehydration.

RNA Extraction

➢ RNA Preparation

- **Filter-based, Spin column Formats**
- Samples are lysed in a buffer that contains **RNase inhibitors** (usually guanidine salts), and **nucleic acids are bound to the membrane** by passing the lysate through the membrane.

- Wash solutions are subsequently passed through the membrane and discarded. An appropriate elution solution is applied and the sample is collected into a tube by centrifugation.

RNA Extraction

- ✓ **DNase digestion**
- ➢ **Storage of isolated RNA**
  - ❖ **Elution**
- ➢ **0.1mM EDTA (in DEPC-treated ultrapure water)**
- ➢ **TE Buffer (10mM Tris-HCl, 1mM EDTA, pH 7.0)**
  - ❖ **Storage**
- ➢ **store RNA at –80°C in single-use aliquots.**

Reverse transcription (RT)

- Is the generation of a complementary strand of DNA (cDNA) from a single strand of RNA.

- **Requirements:**
  - **RNA template**
  - **Primers**
  - **Reverse transcriptase enzyme and buffer**

Reverse transcription (RT)

- **RNA template**
  - **Total RNA or mRNA**

- **Primers**
  - **Oligo (dT)**
  - **Random primers**
  - **Gene specific primer**

- **Reverse transcriptase enzyme**
  - Moloney Murine Leukemia Virus RT (**MMLV)**
  - Avian Myeloblastosis Virus (**AMV**)

Types of RT-PCR

- **One step RT-PCR**

- **Two steps RT-PCR**

Thank You

For your attention

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