

# Mastering RNA Isolation: Choosing the Right Technique for High-Quality Results

## The organic extraction method

Organic extraction of nucleic acids is the most common method for RNA isolation and removing cellular proteins. This technique requires sample homogenization in a phenol-containing solution (usually phenol-chloroform), after centrifugation the samples form two distinct phases. The lower (organic) phase and phase interface contain denatured proteins, while the less-dense upper (aqueous) phase contains nucleic acids, which is then carefully removed by pipetting and RNA is then precipitated with alcohol and rehydrated for further analysis.

## The spin column extraction method

This is a solid phase extraction technique to bind and isolate RNA within filter-based spin columns. These spin columns utilize membranes that contain silica or glass fiber to bind nucleic acids. Samples lysates are passed through the silica membrane using centrifugal force, with the RNA binding to the silica gel at the appropriate pH. The membrane is then washed to remove impurities, with flow-through discarded. RNA is subsequently eluted with RNase-free water, as RNA is stable at a slightly acidic environment.

## Magnetic particle extraction method

This strategy for bioseparation uses magnetic beads coated with, most commonly, a matrix of silica for binding nucleic acids. In this method, lysates incubated with the magnetic beads, allowing the particles to bind RNA molecules.

The magnetic beads are hold in the tube in proximity to an external magnetic field, easily washed of from impurities. The RNA is eluted from the magnetic beads with RNase-free.

	Pros	Cons
Phenol extraction	Gold Standard, well established protocols, RNA is quickly stabilized, applicable to larger/small/difficult samples	Difficult to automate, hazardous chemical, purity process is required
Spin columns	Simple and convenient, amenable to large-scale, high purity	Membranes clog, insufficient yield from difficult samples
Magnetic beads	Most amenable to automation, rapid and simple	Not recommended for viscous samples, laborious when performed manually, risk of contamination of with magnetic beads residual

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