How to isolate good quality nucleic acids

Alt-text Figure 1 - Workflow of an organic extraction protocol

Illustration demonstrating the organic extraction protocol. A source of biological material (e.g. mouse tissue, cell or bacterial culture) is homogenised with a lysis buffer (e.g. guanidium-phenol) in a microtube. This will separate RNA, DNA and Protein from other cell elements. The phase of separation is performed using chloroform. The extraction and precipitation of nucleic acids are carried out using isopropanol washes. The nucleic acid is then resuspended into nuclease-free water or elution buffer.

Alt-text Figure 2 - Diagram showing the steps for viral RNA isolation using a standard commercial kit

Illustration of an extraction workflow using spin-columns. In a microtube, the testing sample and an extraction control aliquot are mixed and incubated. It is recommended to change gloves to prevent downstream contamination. Samples are mixed with ethanol 100% and then, transferred into a spin column. After centrifugation, the flow-through is removed and Wash Buffer 1 is added to the column. Another centrifuge step is performed and Wash Buffer 2 is added. Additional spin steps are performed to remove the buffer excess and dry out the column. The Elution Buffer is added to the column and incubated. The final spin will elute the nucleic acid from the column.

Alt-text Figure 3 - Schematic of RNA isolation using magnetic bead-based technology

Illustration of RNA isolation using magnetic beads. The sample is lysed with an appropriate buffer and magnetic beads are added. The beads will bind to nucleic acids. The sample microtube is placed into a magnetic stand which will attract the beads-nuclei acid complex. The fluid and cell debris are pipetted out of the tube. The microtube is removed from the magnetic stand and the sample is washed with a buffer solution. The sample is placed on the magnetic stand again to remove the solution. The sample is left on the stand to air dry. Once dry, the elution buffer is added to the sample and mixed. The tube is placed on the magnetic stand. The sample elute is then transferred to a clean tube for storage.