4.3 **Column Wash**

1. Apply 500µl of Wash Buffer W3 to the column and centrifuge for 2 min at 5,200 x g.
2. Discard the flow-through and reassemble the spin column with its Collection Tube.
3. Inspect the column to ensure that the liquid has passed through into the Collection Tube. There should be no liquid in the column. If necessary, spin for an additional 1 min at 5,200 x g to dry.

4.4 **Protein Elution and pH Adjustment**

1. Add 9.3µl of Protein Neutralization Buffer to a fresh 1.7ml Elution Tube (supplied).
2. Transfer the spin column from the Column Wash procedure (section 4.3) into the Elution Tube.
3. Apply 100µl of Protein Elution Buffer to the column and centrifuge for 2 min at 5,200 x g to elute bound protein.

   Note: Approximately 95% of bound protein is recovered in the first elution. If desired, a second elution using 50µl of Protein Elution Buffer may be carried out. This should be collected into a different tube (to which 4.6µl of Protein Neutralization Buffer is pre-added) to prevent dilution of the first elution.

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II RNA/DNA/Protein Kit (Phenol free)	50 Preps	BIO-52085

SECTION 1. LYSATE PROTOCOLS

1 To prepare lysate from your starting material, please refer to the supplied manual or download the online protocol (www.bioline.com/isolate).

SECTION 2. GENOMIC DNA PURIFICATION FROM ALL TYPES OF LYSATE

2.1 Binding to DNA Column

1. Assemble an ISOLATE II **DNA Column** (blue ring) with the Collection Tube (supplied).
2. Apply up to 600µl of the lysate prepared from section 1 onto the column and centrifuge for 1 min at 14,000 x g.
   Note: Ensure the entire lysate volume has passed through into the Collection Tube by inspecting the column. If the entire lysate volume has not passed through, spin for an additional 1 min at 14,000 x g.
3. **Retain the flow-through for Total RNA Purification (see section 3).** The flow-through contains the RNA and proteins and should be stored on ice or at -20°C until the Total RNA Purification protocol is carried out.
4. Reassemble the spin column with the Collection Tube

2.2 Genomic DNA Wash

1. Apply 500µl of Wash Buffer W1 to the column and centrifuge for 1 min at 14,000 x g. Discard the flow-through.
2. Apply 500µl of Wash Buffer W2 to the column and centrifuge for 1 min at 14,000 x g. Discard the flow-through.
3. Spin the column for 2 min at 14,000 x g in order to dry the column thoroughly. Discard the Collection Tube.

2.3 DNA Elution

1. Place the column into a fresh 1.7ml Elution Tube (supplied).
2. Add 100µl of DNA Elution Buffer to the column.
3. Centrifuge for 2 min at 200 x g, followed by 1 min at 14,000 x g. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column for an additional 1 min at 14,000 x g.
   Note: For maximum DNA recovery, apply a second volume of 100µl DNA Elution Buffer and repeat step 3. Alternatively, re-apply the first eluate (for higher concentration).

2.4 Storage of DNA

The isolated DNA can be stored at 4°C for up to three days. It is recommended that samples are held at -20°C or -80°C for long term storage.

SECTION 3. TOTAL RNA PURIFICATION FROM ALL TYPES OF LYSATE

3.1 Binding RNA to RNA/Protein Column

1. To every 100µl of flow-through from section 2.1 step 3, add 60µl of 96-100% ethanol. Mix by vortexing.
   Note: For example, for 300µl of flow-through, add 180µl of 96-100% ethanol.
2. Assemble an ISOLATE II **RNA/Protein Column** (black ring) with a Collection Tube (supplied).
3. Apply up to 600µl of the ethanolic lysate onto the column and centrifuge for 1 min at ≥3,500 x g.
   Note: Ensure the entire lysate volume has passed through into the Collection Tube by inspecting the column. If the entire lysate volume has not passed through, spin for an additional 1 min at 14,000 x g.
4. **Retain the flow-through for Protein Isolation (see section 4)**. The flow-through contains the proteins and should be stored on ice or at -20°C until the Protein Isolation protocol is carried out.
5. Depending on your lysate volume, repeat steps 3 and 4 if necessary. The flow-throughs should be combined and retained in the same microcentrifuge tube.
6. Reassemble the spin column with the Collection Tube.
   Optional: For sensitive applications, an optional on-column DNase I treatment can be performed at this point (see full manual Appendix B).

3.2 RNA Wash

1. Apply 400µl of Wash Buffer W1 to the column and centrifuge for 1 min at 14,000 x g.
   Note: Ensure the entire wash buffer volume has passed through into the Collection Tube by inspecting the column. If the entire wash buffer volume has not passed through, spin for an additional 1 min at 14,000 x g.
2. Discard the flow-through and reassemble the column with the Collection Tube.
3. Wash the column a second time by adding another 400µl of Wash Buffer W1 and centrifuge for 1 min at 14,000 x g.
   Note: If performing the optional on-column DNase I treatment (see full manual Appendix B), resume the protocol at this point, by performing this second wash step.
4. Discard the flow-through and reassemble the spin column with its Collection Tube.
5. Wash column a third time by adding 400µl of Wash Buffer W1 and centrifuge for 1 min at 14,000 x g.
6. Discard the flow-through and reassemble the spin column with its Collection Tube.
7. Spin the column for 2 min at 14,000 x g in order to thoroughly dry the column. Discard the Collection Tube.

3.3 **RNA Elution**

1. Place the column into a fresh 1.7ml Elution Tube (supplied).
2. Add 50µl of RNA Elution Buffer to the column.
3. Centrifuge for 2 min at 200 x g, followed by 1 min at 14,000 x g. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column for an additional 1 min at 14,000 x g.
   Note: For maximum RNA recovery, particularly for samples that are known to contain large amounts of RNA, apply a second elution (repeat steps 2 and 3). Alternatively, re-apply the first eluate (for higher concentration).
4. **Retain the column for Protein Isolation (Proceed to section 4).**

3.4 **Storage of RNA**

The isolated RNA can be stored at -20°C for up to three days. It is recommended that samples are held at -80°C for long term storage.

SECTION 4. TOTAL PROTEIN ISOLATION FROM ALL CELL TYPES

4.1 **pH Adjustment of Lysate**

1. Transfer flow-through from the RNA Binding Step (section 3.1 step 4) to a separate 1.5ml microcentrifuge tube (user supplied).
2. For every 100µl of flow-through, add 100µl of molecular biology grade water.
   Note: For example, to purify the entire flow-through of 480µl, add 480µl molecular biology grade water.
3. For every 100µl of flow-through, add 8µl of Protein Binding Buffer. Mix contents well.
   Note: For an entire flow-through of 480µl, add 40µl of Protein Binding Buffer. Depending on the type and amount of input, slight precipitation may occur, which will not affect the purification procedure.

4.2 **Protein Binding**

1. Apply up to 600µl of the pH-adjusted protein sample onto the ISOLATE II **RNA/Protein Column** and centrifuge for 2 min at 5,200 x g. Inspect the column to ensure that the entire sample has passed through into the Collection Tube. If necessary, spin for an additional 3 min at 5,200 x g.
2. Discard the flow-through. Reassemble the spin column with its Collection Tube.
   Note: You can save the flow-through into a fresh tube for assessing the protein's binding efficiency.
3. Depending on your sample volume, repeat steps 1 and 2 until the entire protein sample has been loaded onto the column.