A&A BIOTECHNOLOGY
innovating life science

Manual

Total RNA Zol-Out™

Kit for the rapid purification of ultra-pure total RNA from samples prepared in (TRIzol® TRI Reagent®, RNAzol®, QIAzol®, TriPure ™, TriSure ™, etc.).

catalog #	size
030-25	25 isolations
030-100	100 isolations

Guarantee

A&A Biotechnology provides a guarantee on this kit.
The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- not recommended use of equipment and materials
- the use of other reagents than recommended or which are not a component of the kit
- the use of expired or improperly stored reagents and columns

Table of contents

Kit contents

	25 isolations	100 isolations	storage
Minicolumns for RNA isolation	25 pcs	100 pcs	15–25 °C
2 ml tubes	25 pcs	100 pcs	15–25 °C
A1 wash solution	50 ml	200 ml	15–25 °C
Isopropanol	10 ml	30 ml	15–25 °C
Ultrapure water	8 ml	30 ml	15–25 °C

The binding capacity of the RNA purification column is 100 µg of RNA.

Additional equipment and reagents

Necessary

- 1.5 ml sterile Eppendorf tubes
- Microcentrifuge

Optional

- 96%-99% ethanol
- Chloroform
- BPC (1-bromo-3-chloropropan)
- DNAse (cat. # 1009-10, 1009-100)
- Clean-Up RNA Concentrator (cat. # 039-25C, 039-100C)
- Heatblock or incubator set to 37 °C, 75 °C

Important notes

When working with RNA, use RNAse-free consumables. Work sterile, use disposable gloves and change them whenever good laboratory practice requires it.

Material preparation

For a sample suspended in: TRIzol®, TRI Reagent® and other reagents based on a mixture of phenol and guanidine thiocyanate or hydrochloride (RNAzol®, QIAzol®, TriPure™, TriSure™, etc.)
choose one of four methods (A, B, C or D) to prepare samples for RNA isolation.

To 1 volume of the sample add:

A. **1/5 volume** of **chloroform** (not included) - proportion of 5:1, e.g. add 0.2 ml of chloroform to 1 ml of sample. Follow poin 1. of the protocol.

B. **1/10 volume** of **BCP** (not included) - proportion of 10:1, e.g. add 0.1 ml BPC to 1 ml of sample. Follow poin 1. of the protocol.

C. **1 volume** of **96%-100% ethanol** (not included) - proportion of 1:1, e.g. add 1 ml of ethanol to 1 ml of sample. Follow poin 3. of the protocol.

D. **1/3 volume** of **ultrapure water** (included) - proportion of 3:1, e.g. add 0.3 ml of ultrapure water to 1 ml of sample.

   Centrifuge for **15 min** at **10 000-12 000 RPM**.

   Transfer a supernatant to **a new** 1.5 ml tube (not included).

   Add **1/2 volume** of **isopropanol** (included) - proportion of 2:1, e.g. add 0.5 ml of isopropanol to 1 ml of supernatant.

   Follow poin 3. of the protocol.

Protocol

1. From the prepared material transfer **supernatant** containing RNA to 1.5 ml Eppendorf tube (not included).

2. Add **1/2 volume** of **isopropanol** (included) - proportion of 2:1, e.g. add 300 µl of isopropanol to 600 µl of supernatant.

3. Thoroughly mix and apply 700 µl of the mixture onto the minicolumn.

   Centrifuge for **1 min** at **10 000-12 000 RPM**.

   700 µl of the mixture can be applied onto the mini column at one time. In case of larger volume centrifuge 700 µl of the mixture, apply the rest of the mixture onto the minicolumn and centrifuge again.

4. Transfer the minicolumn to **a new** 2 ml tube (included).

5. Add **700 µl** of the **A1** wash solution.

6. Centrifuge for **1 min** at **10 000-12 000 RPM**.

7. Discard the filtrate from the tube. Place the minicolumn into the same tube.

8. Add **700 µl** of the **A1** wash solution.

9. Centrifuge for **1 min** at **10 000-12 000 RPM**.

10. Discard the filtrate from the tube. Place the minicolumn into the same tube.

11. Add **200 µl** of the **A1** wash solution.

11. Centrifuge for **2 min** at **10 000-12 000 RPM**.

12. Transfer the microcolumn to **a new** 1.5 ml elution tube (included).

13. Add **100 µl** of **ultrapure water**.

14. Keep on **3 min** at **room temp**.

15. Centrifuge for **1 min** at **10 000-12 000 RPM**.

16. Remove the minicolumn, close the elution tube with the purified RNA.
Store at -20, -80°C until later use.

Additional clean-up / concentration of isolated RNA sample (optional)

Total RNA Zol-Out™ kit effectively isolates and purifies RNA for most downstream applications.

In case of the highest possible RNA sample purity being required, as for example supreme DNA removal, we recommend to additionally process RNA sample, as follows:

Use of the DNAse (cat. # 1009-10, 1009-100)

1. **To 100 µl** of **RNA eluate** add:

   1 µl of **DNAse** (10 U/µl)
   10 µl of **10x reaction buffer** (included with DNAse)

2. Incubate for **15 min** at **37 °C**.

3. Incubate for **10 min** at **65 °C** - inactivation of DNAse.

Use of Clean-Up RNA Concentration Kit (cat. # 039-25C, 039-100C)

Kit for removal and concentration of RNA samples. Elution from 15 µl. Microcolums (included with the kit) effectively bind RNA. Most contaminations flow through the microcolumns.
Elution of RNA is performed at 30 µl volume of ultrapure water and enables effective concentration.

Safety Information

DANGER

A1 wash solution

A1 wash solution
H225 Highly flammable liquid and vapor.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

DANGER

Isopropanol

A1 wash solution
H225 Highly flammable liquid and vapor.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

A&A BIOTECHNOLOGY
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A&A Biotechnology, ul. Strzelca 40, 80-299 Gdańsk, Poland
phone +48 883 323 761, +48 600 776 268
info@aabiot.com, www.aabiot.com