A&A BIOTECHNOLOGY
innovating life science

Manual

Total RNA Zol-Out™ D

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

catalog #	size
043-25	25 isolations
043-100	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

Contents

	25 isolations		100 isolations		
component	**quantity**	**cat #**	**quantity**	**cat #**	**storage**
microcolumns	25 pcs	K-C02-25	100 pcs	K-C02-100	15–25 °C
2 ml tubes	25 pcs	K-PC-25	100 pcs	K-PC-100	15–25 °C
1.5 ml elution tubes	25 pcs	K-C01-25P	100 pcs	K-C01-100P	15–25 °C
A2WE wash solution	45 ml	K-A2WE-45	180 ml	K-A2WE-180	15–25 °C
R8I wash solution	20 ml	K-R8I-20	80 ml	K-R8I-80	15–25 °C
DNAse	220 µl	K-DNA-220B	650 µl	K-DNA-650B	-20 °C
10x DNAse buffer	1.5 ml	K-BDNA-15A	1.5 ml	K-BDNA-15A	-20 °C
ultrapure water	8 ml	K-WUP-8	30 ml	K-WUP-30	15–25 °C

The binding capacity of the RNA purification column is 10 µg of RNA.

Additional equipment and reagents

Necessary

- 1.5 ml sterile tubes
- 96-99% ethanol
- microcentrifuge
- heatblock set to 50 °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

1. Add **1/3 volume** of **ultrapure water to 1 volume** of the sample suspended in TRIzol® (e.g. to 300 µl of TRIzol® suspended cells add 100 µl of ultrapure water).
2. Close the tube and mix by inverting the tube a few times.
3. Centrifuge the sample for **10 min** at **10 000 RPM**.
4. Transfer the supernatant to **a new** 1.5 ml tube (not included).
5. Add **1 volume** of 96%-100% **ethanol** (not included) to **1 volume** of the **supernatant** (e.g. add 350 µl of ethanol (96-100%) to 350 µl of the supernatant).
6. Close the tube and mix by inverting the tube a few times.
7. Follow point 1. of the protocol.

DNA digestion solution preparation

Before proceeding to RNA isolation, prepare the appropriate amount of DNA digestion solution according to the following formula (50 µl of solution per sample):

component	volume per 1 sample	volume per 25 samples	volume per 100 samples	n - sample quantity
10x DNAase buffer	5 µl	26 x 5 µl	101 x 5 µl	(n+1) x 5 µl
	+	+	+	+
DNAse	6 µl	26 x 6 µl	101 x 6 µl	(n+1) x 6 µl
	+	+	+	+
ultrapure water	39 µl	26 x 39 µl	101 x 39 µl	(n+1) x 39 µl
	=	=	=	
DNA digestion solution	**50 µl**	**1,3 ml**	**5,05 ml**	

Mix and describe "DNA digestion solution"

Isolation protocol

If sample volume is greater than **700 µl** repeat point 1. and 2. of protocol discarding flow-through after each repetition.

1.	Apply a maximum **700 µl** of prepared material onto the microcolumn.
2.	Centrifuge for **1 min** at **10 000 RPM**.
3.	Apply **700 µl** of the **A2WE** wash solution onto the microcolumn.
4.	Centrifuge for **2 min** at **10 000 RPM**. **Attention**. It is necessary to centrifuge min. 2 min to remove ethanol from the microcolumn membrane.
	On-column DNAse digestion
5.	Transfer the microcolumn to **a new** 2 ml tube (included).
6.	Add **50 µl** of **DNA digestion solution** directly onto the microcolumn membrane, ensuring no droplets remain on the column inner walls or membrane-securing ring.
7.	Incubate for **30 min** at **37 °C**.
8.	Add **700 µl** of **R8I** wash solution.
9.	Centrifuge for **1 min** at **10 000 RPM**.
10.	Collect the filtrate from the tube and apply it again onto the microcolumn. Place the microcolumn into the same tube.
11.	Centrifuge for **1 min** at **12 000 RPM**.
12.	Discard the filtrate from the tube. Place the microcolumn into the same tube.
13.	Add **700 µl** of **A2WE** wash solution.
14.	Centrifuge for **1 min** at **10 000 RPM**.

15. Discard the filtrate from the tube. Place the microcolumn into the same tube.

16. Add **200 µl** of **A2WE** wash solution.

17. Centrifuge for **2 min** at **10 000 RPM**.

    Attention. It is necessary to centrifuge min. 2 min to remove ethanol from the microcolumn membrane.

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

19. Add **40 µl** of **ultrapure water**.

20. Keep for **3 min** at **room temp**.

21. Centrifuge for **1 min** at **10 000 RPM**.

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

Safety information

DANGER

A2WE wash solution

H225 Highly flammable liquid and vapor.
H319 Causes serious eye irritation.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P233 Keep container tightly closed.
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

R8I 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
innovating life science

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

version 2023-2