A&A BIOTECHNOLOGY
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Manual

Total RNA Mini Plus D

Kit for total RNA purification with DNA removal on the column.

catalog #	size
042-25	25 isolations
042-100	100 isolations

For research use only.

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

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

Table of contents

Specification

form	microcolumn
binding capacity	10 µg of RNA
sample size	• bacterial culture: up to 500 µl • yeast culture: up to 500 µl • blood: up to 1 ml • cell culture: up to 5 x 10^5 • plant or animal tissue: up to 10 mg
elution volume	from 15 µl
elution solution	ultrapure water

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
1.5 ml tubes	25 pcs	K-C01-25P	100 pcs	K-C01-100P	15–25 °C
2 ml tubes	50 pcs	K-PC-50	200 pcs	K-PC-200	15–25 °C
DNAse U/µl	220 µl	K-DNA-220B	650 µl	K-DNA-650B	-20 °C
10x DNAase buffer	1.5 ml	K-BDNA-15A	1.5 ml	K-BDNA-15A	-20 °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
Fenozol Plus	15 ml	K-FENP-15	50 ml	K-FENP-50	2–8 °C
Isopropanol	15 ml	K-IZO-15	50 ml	K-IZO-50	15–25 °C
ultrapure water	8 ml	K-WUP-8	30 ml	K-WUP-30	-20–25 °C

Additional equipment and reagents

Necessary

- 1.5 ml sterile tubes
- microcentrifuge
- heatblock or incubator set to 50 °C

Optional

- RBCL (cat. # 213-100, 213-250)

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

Bacterial, yeast culture

1. Centrifuge **100-500 µl** of overnight bacterial culture or yeast culture. Discard supernatants.
2. Follow point 1. of the protocol.

Cell culture

1. Centrifuge cell culture containing **1 x 10^5- 5 x 10^5** of cells. Discard supernatants.
2. Follow point 1. of the protocol.

Plant, animal tissue

1. Homogenize tissue sample **(1-10 mg)** in liquid nitrogen.
2. Transfer the sample into 1.5 ml tube (not included).
3. Follow point 1. of the protocol.

Fresh blood (not frozen)

Additional reagents you will need:

- **RBCL** (max. 5 ml per sample), nr kat. 213-100

1. Add **the appropriate amount** of **RBCL** to **1 ml** of **blood sample**.

   Attention. We recommend using 5 volumes of RBCL to 1 volume of blood sample.

2. Mix and incubate on ice for **15 min**.

   Note. Note the changing appearance of the sample during the incubation. The initially opaque solution should turn to a completely transparent ruby-red at the incubation end.

3. Centrifuge for **10 min** at **3000 x g.**
   Carefully discard supernatants.

4. 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

1. Add **400 µl** of **Fenozol Plus** and pipette until complete lysis cells occurs.

   Attention. Fenozol Plus deactivates endogenous RNAses. Sample suspended in Fenozol Plus can be stored:
   - at -20 °C, -80 °C up to one year
   - from +2 °C to +8 °C up to one week
   - at room temperature up to 24 hours

   Fenozol Plus contains phenol. Avoid contact with skin. Wear suitable protective gloves.

2. Incubate sample for **5 min** at **50 °C**.

3. Add **150 µl** of **ultrapure water**.
   Intensively vortex for **15 s**.

4. Keep the sample for **5 min** at **room temp**.
   Centrifuge the sample for **10 min** at **10 000 RPM**.

   Note. During the centrifugation step,the DNA and proteins are collected at the bottom of the tube while RNA stays dissolved in the supernatant.

5. Transfer **400 µl** of the supernatant to **a new** 1.5 ml tube (not included).
   Add **400 µl** of **isopropanol**.

6. Thoroughly mix and apply onto the microcolumn.

7. Centrifuge for **1 min** at **12 000 RPM**.

8. Transfer the microcolumn to **a new** 2 ml tube (included).
   Add **700 µl** of **A2WE** wash solution.

9. Centrifuge for **2 min** at **12 000 RPM**.

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

On-column DNAse digestion	
10.	Transfer the microcolumn to **a new** 2 ml tube (included).
11.	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.
12.	Incubate for **30 min** at **37 °C**.
13.	Add **700 µl** of **R8I** wash solution.
14.	Centrifuge for **1 min** at **12 000 RPM**.
15.	Collect the filtrate from the tube and apply it again onto the microcolumn. Place the microcolumn into the same tube.
16.	Centrifuge for **1 min** at **12 000 RPM**.
17.	Add **700 µl** of **A2WE** wash solution.
18.	Centrifuge for1 **min at 12 000 RPM**.
19.	Remove the microcolumn from the tube and discard the filtrate. Place the minicolumn into the same tube. Add **200 µl** of **A2WE** wash solution.
20.	Centrifuge for **2 min** at **12 000 RPM**. **Attention**. It is necessary to centrifuge min. 2 min to remove ethanol from the microcolumn membrane.
21.	Transfer the dry microcolumn to **a new** 1.5 ml elution tube (included). Add **15-40 µl** of **ultrapure water** directly onto the minicolumn resin.
22.	Keep for **3 min** at **room temp.** Centrifuge for **1 min** at **10 000-12 000 RPM**.
23.	Remove the **micrcolumn** and store the tube with purified RNA at **-20 °C, -80 °C** until later use. **Note.** Elution tube has a long, elastic cap connector. Start closing the tube by careful pressing the cap on the connector side. A „click" sound confirms proper closure. Different ways of closing may cause opening of the tube during storage.

Safety information

DANGER

Fenozol Plus

H301+H311+H331 Toxic if swallowed, in contact with skin or if inhaled.
H314 Causes severe skin burns and eye damage.
H341 Suspected of causing genetic defects.
H373 May cause damage to organs through prolonged or repeated exposure.
H411 Toxic to aquatic life with long-lasting effects.
P261 Avoid breathing dust.
P273 Avoid release to the environment.
P280 Wear protective gloves, protective clothing, eye protection, face protection.
P301+P310 If swallowed: immediately call a Poison Center or doctor/physician.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a Poison Center or doctor/physician.

DANGER

Isopropanol

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

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.

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.

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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

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