GENETIX BRAND

HANDBOOK

Nucleo-pore®

RNASure® Blood Mini / Midi Kit

☐ RNASure® Blood Mini Kit	NP-74105 (50 Preps)
☐ RNASure® Blood Mini Kit	NP-74107 (250 Preps)
☐ RNASure® Blood Midi Kit	NP-74184 (20 Preps)

www.genetixbiotech.com

Nucleo-pore®

Table of contents

COMPONENTS

Kit contents

	RNASure® Blood Mini Kit	
REF	**50 preps NP-74105**	**250 preps NP-74107**
Lysis Buffer NLB	25 mL	125 mL
Wash Buffer NWB2	13 mL	80 mL
Wash Buffer NWB3 (Concentrate)**	12 mL	3X25 mL
Membrane Desalting Buffer MDSB	25 mL	125 mL
Reaction Buffer for rDNase	7 mL	15 mL
rDNase, RNase-free (lyophilized)**	2 vials (size D)	5 vials (size D)
Liquid Proteinase K	600 µL	3 mL
RNase-free H_2O	13 mL	65 mL
RNASure® Blood Columns (light blue rings -plus Collection Tubes)	50	250
Collection Tubes (2 mL, with lid) for lysis	50	250
Collection Tubes (1.5 mL) for elution	50	250
Collection Tubes (2 mL)	150	750
User manual	1	1

** Please check “Preparation of Reagents”

Kit contents continued

	RNASure® Blood Midi Kit
REF	**20 preps NP-74184**
Lysis Buffer NLB	50 mL
Wash Buffer NWB2	2 × 13 mL
Wash Buffer NWB3 (Concentrate)**	25 mL
Membrane Desalting Buffer MDSB	50 mL
Reaction Buffer for rDNase	7 mL
rDNase, RNase-free (lyophilized)**	2 vials (size D)
Liquid Proteinase K	800 µL
RNase-free H_2O	13 mL
RNASure® Blood Midi Columns (plus Collection Tubes)	20
Collection Tubes (15 mL) for lysis, elution, and washing steps	60
User manual	1

** Please check “Preparation of Reagents”

Reagents, consumables, and equipment to be supplied by user

Reagents

- 96 – 100 % ethanol (to prepare Wash Buffer NWB3)
- 70 % ethanol (to adjust RNA binding conditions)

Consumables

- Sterile RNase-free tips

Equipment

- Manual pipettors
- Vortex mixer
- Centrifuge for 15 mL tubes with a swing-out rotor capable of reaching 4,500 x g
- Centrifuge for microcentrifuge tubes (RNASure Blood)
  (RNASure ® Blood Midi)
- Personal protection equipment (e.g., lab coat, gloves, goggles)

INTRODUCTION

Principle & Procedure

The **RNASure® Blood** kits offer a direct total blood lysis from 200 – 400 µL (RNASure® Blood Mini kit) or 400 – 1300 µL (RNASure Blood Midi Kit) whole blood collected in standard (e.g., EDTA) blood collection tubes. One of the most important aspects in RNA purification is to prevent RNA degradation during the isolation. With the **RNASure® Blood** isolation kit, leukocytes (the main source of RNA in whole blood) and other blood cells, are lysed by incubating the whole blood in a solution containing large amounts of chaotropic ions. This lysis buffer immediately inactivates RNases (which are present in virtually all biological materials) and creates appropriate binding conditions that favor adsorption of RNA to the silica membrane. A complex selective erythrocyte lysis and preparation of a leukocyte pellet is not necessary. Contaminating DNA, which is also bound to the silica membrane, is removed by a recombinant DNase solution (supplied) which is directly applied onto the silica membrane during the preparation. Simple washing steps with two different buffers remove salts, metabolites, and macromolecular cellular components. Pure RNA is finally eluted under low ionic strength conditions with RNase-free H_2O (supplied).

The RNA preparation using **RNASure® Blood** kits is performed at room temperature. A refrigerated centrifuge is not necessary. The eluate, however, should be handled with care because RNA is very sensitive to trace contaminations of RNases, often found on general labware, fingerprints, and dust. To ensure RNA stability, keep RNA frozen at -20ºC for short-term or -70ºC for long-term storage.

Kit specifications

- **RNASure® Blood** kits are recommended for the isolation of RNA from whole blood (e.g., stabilized with EDTA, citrate, or heparin).
- The **RNASure® Blood** kits allow the purification of RNA with an A_{260} / A_{280} ratio typically exceeding 1.9 (measured in TE buffer, pH 7.5).
- The isolated RNA is ready to use for typical downstream applications (e.g., reverse transcriptase-PCR (RT-PCR)).
- RNA isolated with the RNASure® kits is typically of high integrity. However, RNA integrity strongly depends on the sample quality.
- The amount of DNA contamination is significantly reduced during on-column digestion with rDNase. However, in very sensitive applications, it may be possible to detect traces of DNA. The probability of DNA detection with PCR increases with:
  1. the number of DNA copies per preparation: single copy target < plastidial / mitochondrial target < plasmid transfected into cells.
  2. decreasing PCR amplicon size.

Table 1: Kit specifications at a glance

Parameter	RNASure® Blood Mini Kit	RNASure® Blood Midi Kit
Sample material	200 – 400 µL fresh or frozen whole blood (e.g., stabilized with EDTA, citrate, or heparin)	400 – 1300 µL fresh or frozen whole blood (e.g., stabilized with EDTA, citrate, or heparin)
Format	Mini spin column	Midi spin column
Fragment size	> 200 nt	> 200 nt
Typical yield	~ 7 µg (3 – 20 µg) per 1 mL blood from healthy subjects	~ 7 µg (3 – 20 µg) per 1 mL blood from healthy subjects
A_{260}/A_{280}	1.9 – 2.1	1.9 – 2.1
Elution volume	40 – 120 µL	200 – 400 µL
Binding capacity	200 µg	700 µg
Preparation time	55 min/6 preps (excl. lysis)	75 min/6 preps (excl. lysis)

The **RNASure® Blood** mini kit contains one protocol that allows the use of 200 µL of whole blood by a total direct blood lysis and a second protocol for processing 400 µL of whole blood with a second loading step.

The RNASure® Blood Midi kit contains a protocol that allows 1.3 mL of whole blood by a total direct blood lysis.

If other volumes than 200 µL, 400 µL, or 1300 µL blood are used, adjust the volumes of Buffer NLB and 70 % ethanol in step 1 and 2 of the corresponding protocol by maintaining the following ratio:

1:1:1 (sample / Buffer NLB / 70 % ethanol)
Example: 300 µL blood +300 µL Buffer NLB +300 µL 70 % ethanol

The volume of Proteinase K can be calculated as follows:
Blood volume µL / 40 = volume Proteinase K µL
Example: 300 µL blood / 40 = 7.5 µL Liquid Proteinase K

The isolated RNA can be used as a template in RT-PCR-reactions. Generally, 1– 40 % of the eluate from RNA prepared with 200 – 400 µL blood is suitable as a template for RT-PCR. If possible, intron-spanning primers should be used for RT-PCR.

Handling, preparation, and storage of starting materials

RNASure® Blood kits are designed for isolation of total RNA from fresh, human whole blood. Whole blood should be collected in the presence of an anticoagulant, preferably EDTA, citrate, or heparin.

It is highly recommended to process blood samples within a few hours after collecting them (when EDTA, citrate, or heparin collection tubes are used). Samples should be stored at 4°C for no longer than 24 hours. The mRNAs contained in blood cells have different stabilities. As a result, in order to ensure that the isolated RNA contains a representative distribution of mRNAs, blood samples should not be stored for long periods before isolating RNA.

If frozen blood samples have to be processed, aliquots of 200 µL, 400 µL, or 1300 µL, preferably, of frozen blood aliquots should be quickly thawed in the presence of 1 volume Lysis Buffer NLB while shaking.

If intermediate storage of stabilized whole blood is necessary, it is recommended storing the lysates at -20 °C. For this, add the indicated volume of Lysis Buffer NLB to the blood sample without adding Liquid Proteinase K. Store the lysates at -20°C. After thawing, add Liquid Proteinase K and follow the protocol at step 1.

Wear gloves at all times during the preparation. Change gloves frequently

Elution procedures

It is possible to adjust the elution method and volume of RNase-free water used for the subsequent application of interest (refer to Table 1 regarding suitable ranges of elution volumes). In addition to the standard method described in the individual protocols (recovery rate about 70 – 90 %), there are several modifications possible:

- **High yield:** Perform two elution steps with the volume indicated in the individual protocol. About 90 – 100 % of bound nucleic acids will be eluted.
- **High yield and high concentration:** Elute with the standard elution volume and apply the eluate once more onto the column for re-elution.

Eluted RNA should be immediately placed and kept on ice for optimal stability and to prohibit omnipresent RNases (general lab ware, fingerprints, dust) from degrading the RNA. For short term storage, freeze at -20 °C, for long term storage, freeze at -70 °C.

Storage conditions and preparation of Reagents

Attention: Buffers NLB, NWB2, and MDSB contain chaotropic salts. Wear gloves and goggles!

CAUTION: Buffers NLB , NWB2, and MDSB contain guanidinium salts which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

- For optimal stability (up to 1 year) it is recommended to store the lyophilized **rDNase (RNase-free)** at 4 °C upon arrival.
- Lysis Buffer NLB is light sensitive during long time storage. Therefore, Lysis Buffer NLB is provided in a black bottle. Short light exposure (several hours) does not affect the buffer.
- After first use, it is recommended to store Liquid Proteinase K at 4°C or -20 °C.
- All other kit components should be stored at 15 – 25 °C and are stable until: see package lable. Storage at lower temperatures may cause precipitation of salts.
- Check that 70 % ethanol is available as additional solution to adjust RNA binding conditions.
- Check that 96 – 100 % ethanol is available as additional solution to prepare Wash Buffer NBW3.

Before starting any **RNASure® Blood** protocol, prepare the following:

- **rDNase (RNase-free)**: Add indicated volume of Reaction Buffer for rDNase (see table below) to the rDNase vial and incubate for 1 min at room temperature. Gently swirl the vials to completely dissolve the rDNase. Be careful not to mix rDNase vigorously as rDNase is sensitive to mechanical agitation. Dispense into aliquots and store at -20 °C. The frozen working solution is stable for 6 months. Do not freeze / thaw the aliquots more than three times. (Be careful when opening the vial as some particles of the lyophilisate may be attached to the lid.)
- **Wash Buffer NWB3**: Add the indicated volume of 96 – 100 % ethanol (see table below) to Buffer NBW3 Concentrate. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer NBW3 at 15 – 25 °C for up to one year.

	RNASure® Blood Mini Kit		**RNASure® Blood Midi Kit**
REF	50 preps NP-74105	250 preps NP-74107	20 preps NP-74184
Wash Buffer NWB3 Concentrate	12 mL Add 48 mL ethanol	3 X 25 mL Add 3 X 100 mL ethanol	25 mL Add 100 mL ethanol
rDNase, RNase-free (lyophilized)	2 vials (size D) Add 2.5 mL Reaction Buffer for rDNase to each vial	5 vials (size D) Add 2.5 mL Reaction Buffer for rDNase to each vial	2 vials (size D) Add 2.5 mL Reaction Buffer for rDNase to each vial

PROTOCOLS

RNASure® Blood Mini protocols

RNA isolation from 200 µL blood

Before starting the preparation:

- Check if Wash Buffer NWB3 and rDNase were prepared according to “preparation of reagents”.
- The complete procedure should be performed at room temperature (18 – 25 °C).
- The use of blood collected in common blood collection tubes with anticoagulant (typically EDTA) is recommended. For frozen blood samples.
- Check if 70 % ethanol is available to adjust binding conditions.

1 **Lyse blood**

Take **200 µL whole blood** in a Collection Tube (2 mL, with lid; provided).

200 µL blood

Add **200 µL Lysis Buffer NLB** to the tube and close the lid. Mix. If necessary, shortly spin to clean the lid.

+200 µL NLB

Add **5 µL Liquid Proteinase K** and close the lid.

+5 µL Liquid Proteinase K

Incubate **3 – 15 min** at **room temperature** (18 – 25 °C) vigorously shaking the tube on a shaker

RT, 3 – 15 min

Centrifuge briefly to clean the lid (**~ 1** s at **~ 2,000 x** g). Short spin only!

~ 2,000 x g, ~ 1 s

Note: Mixtures of blood and Lysis Buffer NLB can be stored for up to five days at +4 °C or up to 2 weeks at -20 °C or below largely maintaining RNA quality and yield in the subsequent purification. After such an intermittend storage continue with addition of Liquid Proteinase K and incubation for 3 – 15 minutes at room temperature. Do not add proteinase K before such an intermittend storage!

2 **Adjust RNA binding conditions**

Add **200 µL 70 % ethanol** to the tube and mix vigorously.

+200 µL 70 % ethanol

Mix

Note: It is important to thoroughly mix the ethanol with the lysate. Recommended: Place tubes in a rack with lid. Close the rack lid and strongly shake the assembly. Alternatively, pipette the solution up and down ~ 5 times.

Centrifuge briefly to clean the lid (**~ 1 s** at **~ 2,000 x** g). Short spin only!

~ 2,000 x g, ~ 1 s

3 **Bind RNA**

Adjust pipette to **610 µL** and transfer lysate into a **RNASure® Blood Column** placed in a Collection Tube.

Load lysate

Note: Do not pipette more than 650 µL into the spin column, this will cause the column to overflow! Avoid formation of foam and aerosols! Avoid wetting the rim (edge) of the column.

Centrifuge **30 s** at **11,000 x** g. Discard flow-through and Collection Tube. Place the column in a new Collection Tube (2 mL; provided).

11,000 x g, 30 s

Note: Mixtures of blood and Lysis Buffer NLB can be stored for up to five days at +4 °C or below largely maintaining RNA quality and yield in the subsequent purification. After such an intermittend storage continue with addition of Liquid Proteinase K and incubation for 3 – 15 minutes at room temperature. Do not add proteinase K before such an intermittend storage!

4 **Desalt silica membrane**

Add **350 µL MDSB** (Membrane Desalting Buffer) onto the column and centrifuge **30 s** at **11,000 x** g.

+350 µL MDSB

11,000 x g, 30 s

Note: After centrifugation, the column can remain in the Collection Tube including the flow-through! The flow-through may be slightly brown. The flow-through can remain in the tube without disturbing DNA digestion.

5 **Digest DNA**

Add **95 µL rDNase** onto the column. Incubate at **room temperature** for **15 min**.

+95 µL rDNase

RT, 15 min

Note: Centrifugation after incubation is not necessary.

6 **Wash and dry silica membrane**

1st wash

+200 µL NWB2

Add **200 µL Buffer NWB2** to the RNASure® Blood Column. Centrifuge for **30 s** at **11,000 x** g. Discard flow-through and Collection Tube and place the column into a new Collection Tube (2 mL; provided).

11,000 x g, 30 s

Buffer NWB2 will inactivate the rDNase.

2nd wash

+600 µL NWB3

Add **600 µL Buffer NBW3** to the RNASure® Blood Column. Centrifuge for **30 s** at **11,000 x** g. Discard flow-through and place the column into a new Collection Tube (2 mL; provided).

11,000 x g, 30 s

Note: Make sure that residual buffer from the previous steps is washed away with Buffer NBW3, especially if the lysate has been in contact with the inner rim of the column during loading of the lysate onto the column. For efficient washing of the inner rim flush it with Buffer NBW3.

3rd wash

+250 µL NWB3

Add **250 µL Buffer NBW3** to the RNASure® Blood Column. Centrifuge for **2 min** at **11,000 x** g. In this step, ethanol is removed from the column.

11,000 x g, 2 min

Place the column into a nuclease-free Collection Tube (1.5 mL, supplied) and discard the Collection tube with flow-through from the previous step.

If for any reason the liquid level in the Collection Tube has reached the RNASure® Blood Column after centrifugation, discard flow-through, and centrifuge again.

7 **Elute RNA**

60 µL RNase-free H_2O

Add **60 µL RNase-free H_2O** (supplied) onto the column and centrifuge **30 s** at **11,000 x** g. The RNA is eluted into the Collection Tube.

11,000 x g, 30 s

RNA isolation from 400 µL blood

Before starting the preparation:

- Check if Wash Buffer NWB3 and rDNase were prepared according to Preparation of Reagents.
- The complete procedure should be performed at room temperature (18 – 25 °C).
- The use of blood collected in common blood collection tubes with anticoagulant (typically EDTA) is recommended.
- Check if 70 % ethanol is available to adjust binding conditions.

1 Lyse blood

Take **400 µL whole blood** in a Collection Tube (2 mL, with lid; provided).

400 µL blood

Add **400 µL Lysis Buffer NLB** to the tube and close the lid. Mix. If necessary, shortly spin to clean the lid.

+400 µL NLB

Add **10 µL Liquid Proteinase K** and close the lid.

+10 µL Liquid Proteinase K

Incubate **3 – 15 min** at **room temperature** (18 – 25 °C) vigorously shaking the tube on a shaker.

RT, 3 – 15 min

Centrifuge briefly to clean the lid (**~ 1** s at **~ 2,000 x** g). Short spin only!

~ 2,000 x g, ~ 1 s

Note: Mixtures of blood and Lysis Buffer NLB can be stored for up to five days at +4 °C or up to 2 weeks at -20 °C or below largely maintaining RNA quality and yield in the subsequent purification. After such an intermittend storage continue with addition of Liquid Proteinase K and incubation for 3 – 15 minutes at room temperature. Do not add proteinase K before such an intermittend storage!

2 Adjust RNA binding conditions

Add **400 µL 70 % ethanol** to the tube and mix vigorously.

+400 µL 70 % ethanol

Mix

Note: It is important to thoroughly mix the ethanol with the lysate. Recommended: Place tubes in a rack with lid. Close the rack lid and strongly shake the assembly. Alternatively, pipette the solution up and down ~ 5 times.

Centrifuge briefly to clean the lid (**~ 1 s** at **~ 2,000 x** g). Short spin only!

~ 2,000 x g, ~ 1 s

3 Bind RNA

Transfer **610 µL** and transfer lysate into a **RNASure® Blood Column** placed in a Collection Tube.

Load 610 µL lysate

Note: Do not pipette more than 650 µL into the spin column, this will cause the column to overflow! Avoid formation of foam and aerosols! Avoid wetting the rim (edge) of the column.

Centrifuge **30 s** at **11,000 x** g. Discard flow-through and Collection Tube. Place the column in a new Collection Tube (2 mL; provided).

11,000 x g, 30 s

Apply the remaining lysate into the RNASure® Blood Column.

Load residual lysate

Note: Do not pipette more than 650 µL into the spin column, this will cause the column to overflow! Avoid foam and aerosol formation! Avoid wetting the rim (edge) of the column.

Centrifuge **30 s** at **11,000 x** g. Discard flow-through and Collection Tube. Place the column in a new Collection Tube (2 mL; provided).

11,000 x g, 30 s

4 Desalt silica membrane

Add **350 µL MDSB** (Membrane Desalting Buffer) onto the column and centrifuge **30 s** at **11,000 x** g.

+350 µL MDSB

Note: After centrifugation, the column can remain in the Collection Tube including the flow-through! The flow-through may be slightly brown. The flow-through can remain in the tube without disturbing DNA digestion.

11,000 x g, 30 s

5 Digest DNA

Add **95 µL rDNase** onto the column. Incubate at **room temperature** for **15 min**.

+95 µL rDNase

RT, 15 min

Note: Centrifugation after incubation is not necessary.

6 Wash and dry silica membrane

1st wash

Add **200 µL Buffer NWB2** to the RNASure® Blood Column. Centrifuge for **30 s** at **11,000 x** g. Discard flow-through and Collection Tube and place the column into a new Collection Tube (2 mL; provided).

+200 µL NWB2

11,000 x g, 30 s

Buffer NWB2 will inactivate the rDNase.

2nd wash

Add **600 µL Buffer NWB3** to the RNASure® Blood Column. Centrifuge for **30 s** at **11,000 x** g. Discard flow-through and place the column into a new Collection Tube (2 mL; provided).

+600 µL NWB3

11,000 x g, 30 s

<u>Note:</u> Make sure that residual buffer from the previous steps is washed away with Buffer NWB3, especially if the lysate has been in contact with the inner rim of the column during loading of the lysate onto the column. For efficient washing of the inner rim flush it with Buffer NWB3.

3rd wash

Add **250 µL Buffer NWB3** to the RNASure® Blood Column. Centrifuge for **2 min** at **11,000 x** g. In this step, ethanol is removed from the column.

+250 µL NWB3

11,000 x g, 2 min

Place the column into a nuclease-free Collection Tube (1.5 mL, supplied) and discard the Collection tube with flow-through from the previous step.

If for any reason the liquid level in the Collection Tube has reached the RNASure® Blood Column after centrifugation, discard flow-through, and centrifuge again.

7 Elute RNA

Add **60 µL RNase-free H_2O** (supplied) onto the column and centrifuge **30 s** at **11,000 x** g. The RNA is eluted into the Collection Tube.

60 µL RNase-free H_2O

11,000 x g, 30 s

RNASure® Blood Midi protocol – RNA isolation from 1.3 mL blood

Before starting the preparation:

- Check if Wash Buffer NWB3 and rDNase were prepared according to preparation of reagents.
- The complete procedure should be performed at room temperature (18 – 25 °C).
- The use of blood collected in common blood collection tubes with anticoagulant (typically EDTA) is recommended.
- Check if 70 % ethanol is available to adjust binding conditions.
- For centrifugation, a centrifuge with a swing-out rotor and appropriate buckets capable of reaching 4,500 x g is required.

1 Lyse blood

1.3 mL blood

Take **1.3 mL whole blood** in a 15 mL tube (provided).

+1.3 mL NLB

Add **1.3 mL Lysis Buffer NLB** to the tube and close the lid. Mix. If necessary, shortly spin to clean the lid.

+33 µL Liquid Proteinase K

Add **33 µL Liquid Proteinase K** and close the lid.

RT, 3 – 15 min

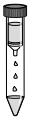
Incubate **3 – 15 min** at **room temperature** (18 – 25 °C) vigorously shaking the tube.

~ 2,000 x g, ~ 1 s

Centrifuge briefly to clean the lid (**~ 1 s** at **~ 2,000 x** g). Short spin only!

Note: Mixtures of blood and Lysis Buffer NLB can be stored for up to five days at +4 °C or up to 2 weeks at -20°C or below largely maintaining RNA quality and yield in the subsequent purification. After such an intermittend storage continue with addition of Liquid Proteinase K and incubation for 3 – 15 minutes at room temperature. Do not add proteinase K before such an intermittend storage!

2 Adjust RNA binding conditions



+1.3 mL 70 % ethanol

Mix

Add **1.3 mL 70 %** ethanol to the tube and mix vigorously.

Note: It is important to thoroughly mix the ethanol into the lysate. Recommended: Vigorously shake for 5 s (e.g., on a vortexer at medium speed). Alternatively, pipette the solution up and down ~ 5 times.

~ 2,000 x g, ~ 1s

If necessary, centrifuge briefly to clean lid (**~ 1s** at **~ 2,000 x** g). Short spin only!

This centrifugation step can be omitted if the lid is not wetted by the lysate. For example, mix by vortexing at medium speed or by pipetting up and down.

3 Bind RNA

Transfer the complete lysate **(~ 4000 µL)** into a RNASure® Blood Midi Column placed in a 15 mL Collection Tube.

Do not pipette more than 4000 µL into the Midi column, this will cause the column to overflow! Avoid foam and aerosol formation!

Centrifuge **3 min** at **4,500 x** g and leave the column in the tube with the flow-through.

Load max. 4000 µL lysate

4,500 x g, 3 min

4 Desalt silica membrane

Add **1.2 mL MDSB (Membrane Desalting Buffer)** onto the column and centrifuge **3 min** at **4,500 x** g.

Discard flow-through and Collection Tube and place the column in a new Collection Tube (15 mL, provided).

+1.2 mL MDSB

4,500 x g, 3 min

5 Digest DNA

Add **240 µL rDNase** onto the column.

Incubate at **room temperature** for **15 min** (centrifugation after this incubation is not necessary).

+240 **µL** rDNase

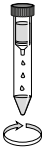
RT, 15 min

6 Wash and dry silica membrane

1st wash

Add **1 mL Buffer NWB2** to the RNASure® Blood Midi Column. Centrifuge for **3 min** at **4,500 x** g.

Leave the RNASure® Blood Midi Column in the tube with the flow-through.



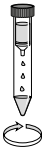
+1 mL NWB2

4,500 x g, 3 min

2nd wash

Add **3 mL Buffer NWB3** to the RNASure® Blood Midi Column. Centrifuge for **3 min** at **4,500 x** g.

Place the column into a nuclease-free Collection Tube (15 mL; provided) and discard the Collection Tube with flow-through from the previous step.



+3 mL NWB3

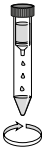
4,500 x g, 3 min

Caution! Ethanol carry-over may impair downstream analysis (e.g., C_p retardation)! Therefore, removal of the column tube assembly from the centrifuge, transport to the lab bench, placement of it onto the bench and removal of the column from the tube should be done with great care in order to avoid any contamination of the column outlet with the flow-through/ethanol. To avoid any risk of wash buffer/ ethanol contamination of the sample, discard wash buffer flow-through from the tube, re-insert the column into the tube and perform a further centrifugation (1 – 3 min 4,500 x g) in order to securely dry the column. Alternatively, use a fresh collection tube (not provided) for a secure column drying centrifugation.

7 Elute RNA

Add **200 µL RNase-free H_2O** (supplied) onto the column. Centrifuge for **3 min** at **4,500 x** g . The RNA is eluted into the Collection Tube.

An additional elution step with **200 µL** fresh elution buffer will increase the total yield by approximately 25%.



+200 µL RNase-free H_2O

4,500 x g, 3 min

Appendix

rDNase digestion in solution

The on-column rDNase digestion in the standard protocol is already very efficient and results in minimal residual DNA. This DNA will not be detectable in most downstream applications. Despite this, there are still certain applications which require even lower contents of residual DNA. However, removal of DNA to a completely undetectable level is challenging and the efficiency of an on-column DNA digestion is sometimes not sufficient for downstream applications requiring lowest residual content of DNA.

A typical example for such a demanding application is an RT-PCR reaction in which the primer molecules do not differentiate between cDNA (derived from RNA) and contaminating genomic DNA. Especially, if

- high copy number targets are analyzed (e.g., multi gene family, mitochondrial, plastidal or plasmid targets (from transfections))
- the target gene is of a very low expression level
- the amplicon is relatively small (< 200 bp).

DNA digestion in solution can efficiently destroy contaminating DNA. However, stringent RNase control and subsequent re-purification of the RNA (in order to remove buffer, salts, rDNase, and digested DNA) are usually required.

The high quality, recombinant, RNase-free DNase (rDNase) in the SureSpin RNA Blood kits can also be used for a digestion in solution in order to remove trace amounts of contaminating DNA.

1 **Digest DNA (Reaction setup)**

Add **0.5 µL rDNase** per **10 µL eluted RNA** and mix moderately.

Centrifuge briefly (**~ 1 s** at **~ 2,000 x** g) to collect all liquid in the lower part of the tube.

Note: This step is important to ensure that every droplet of the RNA comes into contact with the rDNase to ensure efficient DNA digestion.

2 **Incubate sample**

Incubate for **10 min** at **37 °C**.

3 **Repurify RNA**

Repurify RNA with a suitable RNA cleanup procedure, for example, using the SureTrap RNA Clean-up kit (see ordering information) or by ethanol precipitation.

Ethanol precipitation, exemplary:

Add **0.1 volume** of **3 M sodium acetate**, **pH 5.2** and **2.5 volumes of 96 – 100 % ethanol** to **one volume of sample**. Mix thoroughly.

Incubate **several minutes** to **several hours** at **-20 °C** or **4 °C**.

Note: Choose long incubation times if the sample contains low RNA concentration. Short incubation times are sufficient if the sample contains high RNA concentration.

Centrifuge for **10 min** at **maximum speed**.

Wash RNA pellet with **70 % ethanol**.

Dry RNA pellet and resuspend RNA in RNase-free H_2O.

TROUBLESHOOTING GUIDE

RNA is degraded / no RNA obtained

Possible cause

- RNase contamination

Suggestion(s)

- Create an RNase-free working environment. Wear gloves during all steps of the procedure. Change gloves frequently. Use of sterile, disposable polypropylene tubes is recommended. Keep tubes closed whenever possible during the preparation. Glassware should be oven-baked for at least 2 hours at 250 °C before use.

Poor RNA quality or yield

Possible cause

- Reagents not applied or restored properly

Suggestion(s)

- Reagents not properly restored. Add the indicated volume of Reaction Buffer for rDNase and 96 % ethanol to Buffer NWB3 Concentrate and mix. Reconstitute and store lyophilized rDNase according to instructions given in preparation and reagents.
- Sample and reagents have not been mixed completely. Always vortex vigorously after each reagent has been added.
- No ethanol has been added after lysis. Binding of RNA to the silica membrane is only effective in the presence of ethanol.

Possible cause

- Kit storage

Suggestion(s)

- Reconstitute and store lyophilized rDNase according to instructions given in preparation and reagents.
- Store other kit components at room temperature. Storage at low temperatures may cause salt precipitation.
- Keep bottles tightly closed in order to prevent evaporation or contamination.

Possible cause

- Ionic strength and pH influence A260 absorption as well as ratio A260 / A280

Suggestion(s)

- For adsorption measurement, use 5 mM Tris pH 8.5 as diluent.

Clogged Nucleopore® Column / Poor RNA quality or yield

Possible cause

- Sample material

Suggestion(s)

- Bad sample quality. Make sure blood is collected into a standard blood collection tube (e.g., EDTA tube) according to the manufacturer's instructions; using fresh blood is always recommended. Sample should be stored at 4 °C for no longer than 24 hours. Freeze sample if it is not possible to process within one day.

Possible cause

- Inappropriate lysis / binding conditions

Suggestion(s)

- Do not premix Liquid Proteinase K with Lysis Buffer NLB.
- Make sure to vigorously shake during lysis incubation – shaking is essential for the procedure!
- Make sure to use 70 % ethanol in this procedure to adjust binding conditions.

Contamination of RNA with genomic DNA

Possible cause

- rDNase not active

Suggestion(s)

- Reconstitute and store lyophilized rDNase according to instructions given in preparation & reagents.

Possible cause

- DNase solution not properly applied

Suggestion(s)

- Pipette rDNase solution directly onto the center of the silica membrane.

Possible cause

- High leukocyte number

Suggestion(s)

- The higher the leukocyte number, the higher the risk to detect residual DNA in the eluted RNA. To avoid this, use less blood.

Possible cause

- DNA detection system too sensitive

Suggestion(s)

- The amount of DNA contamination is effectively reduced during the on-column digestion with rDNase. However, it cannot be guaranteed that the purified RNA is 100 % free of DNA. Therefore, in very sensitive applications it might still be possible to detect DNA. The Nucleopore® RNA system was checked by the following procedure: One million HeLa cells are subjected to RNA isolation. RNA eluate is used as a template for PCR detection of a 1 kb fragment in a 30 cycle reaction. Generally, no PCR product is obtained while skipping the DNase digest usually leads to positive PCR results.

Suggestion(s)

- The probability of DNA detection with PCR increases with: -the number of DNA copies per preparation: single copy target < plastidial / mitochondrial target < plasmid transfected into cells
  -decreasing of PCR amplicon size.
- Use larger PCR targets (e.g., > 500 bp) or intron spanning primers if possible.

Use protocol 7.1 for subsequent rDNase digestion in solution

Suboptimal performance of RNA in downstream experiments

Possible cause

- Carry-over of ethanol or salt

Suggestion(s)

- Do not let the flow-through touch the column outlet after the second Buffer NWB3 wash. Be sure to centrifuge at the corresponding speed for the respective time in order to remove ethanolic Buffer NWB3 completely.
- Check if Buffer NWB3 has been equilibrated to room temperature before use. Washing at lower temperatures lowers efficiency of salt removal by Buffer NWB3.

Possible cause

- Store isolated RNA properly

Suggestion(s)

- Eluted RNA should always be kept on ice for optimal stability since trace contaminations of omnipresent RNases (general lab ware, fingerprints, dust) will degrade the isolated RNA. For short term storage freeze at -20 °C, for long term storage freeze at -70 °C.

ORDERING INFORMATION

Description	Pack Size	Cat. No.
DNASure® Tissue Mini Kit	50 preps	NP-61305
DNASure Plant Mini Kit	50 preps	NP-79105
DNASure Plant Mini Kit	250 preps	NP-79107
DNASure Plant Midi Kit	20 preps	NP-78153
DNASure Plant Maxi Kit	10 preps	NP-78164
DNASure Blood Mini Kit	50 preps	NP-61105
DNASure Blood Mini Kit	250 preps	NP-61107
DNASure Blood Midi Kit	20 preps	NP-61184
DNASure Blood Maxi Kit	10 preps	NP-61193
DNASure Blood FastPure Kit	50 preps	NP-62205
DNASure Blood FastPure Kit	250 preps	NP-62207
SureSpin Plasmid Mini Kit	50 preps	NP-37105
SureSpin Plasmid Mini Kit	250 preps	NP-37107
SureSpin Plasmid FastPrep Kit	50 preps	NP-47105
SureSpin Plasmid FastPrep Kit	250 preps	NP-47107
SureSpin Buffer Set*	1	37107-BS
SurePrep Plasmid Mini Kit	20 preps	NP-15123
SurePrep Plasmid Mini Kit	100 preps	NP-15125
SurePrep Plasmid Midi Kit	20 preps	NP-15143
SurePrep Plasmid Midi Kit	100 preps	NP-15145
SurePrep Plasmid Maxi Kit	10 preps	NP-15161
SurePrep Plasmid Maxi Kit	25 preps	NP-15162
SurePrep Plasmid Mega Kit	5 preps	NP-15183
SurePrep Plasmid Giga Kit	5 preps	NP-15191

***SureSpin® Buffer Set**

For the isolation of low-copy plasmids, buffers PA1, PA2, PA3, RNase A, sufficient for 300 preps

ORDERING INFORMATION

Description	Pack Size	Cat. No.
SurePrep® Buffer Set**	1	15143-BS
SurePrep® Plasmid Endofree Maxi Kit	10 preps	NP-15363
SurePrep Plasmid Endofree Mega Kit	5 preps	NP-15365
SurePrep® Plasmid Endofree Giga Kit	5 preps	NP-15367
SureSpin® 96 PCR Kit	4x96	NP-38151
SureTrap® Gel Extraction Kit	50 preps	NP-38705
SureTrap® Gel Extraction Kit	250 preps	NP-38707
SureTrap® PCR Cleanup Kit	50 preps	NP-38105
SureTrap® PCR Cleanup Kit	250 preps	NP-38107
SureExtract® Spin PCR/Gel Extraction Kit	50 preps	NP-36105
SureExtract® Spin PCR/Gel Extraction Kit	250 preps	NP-36107
SureSEQ® Cleanup Kit	50 preps	NP-73205
RNASure® Mini Kit	50 preps	NP-84105
RNASure® Mini Kit	250 preps	NP-84107
RNASure® Plant Kit	50 preps	NP-84905
RNASure® Plant Kit	250 preps	NP-84907
miRNASure® Mini Kit	50 preps	NP-71002
SureTrap® mRNA Mini Kit	12 preps	NP-80033
SureTrap® mRNA Midi Kit	12 preps	NP-80043
RNASure® Virus Kit	50 preps	NP-67705
RNASure® Virus Kit	250 preps	NP-67707

**SureSpin® Buffer Set

For isolation of low-copy plasmids, cosmids, BACs, PACs, and P1 constructs, only applicable with SurePrep® Plasmid kits, sufficient for 10 SurePrep Maxi Columns (Maxi preps), 20 SurePrep® Midi Columns (Midi preps), set incl. RNase A

ORDERING INFORMATION

Description	Pack Size	Cat. No.
Nucleo-pore® Stool DNA Mini Kit	50	NP-7011D
Nucleo-pore® gRNA Blood Kit	50	NP-0201R
Nucleo-pore® gDNA Urine Kit	20	NP-6030D
Nucleo-pore® Yeast Transformation Kit	120	NP-1002T
Nucleo-pore® DNA Methylation Kit	50	NP-6006D
Nucleo-pore® gDNA Clean-up Kit	200	NP-4304D
Nucleo-pore® Bisulphite DNA Clean-up Kit	50	NP-5205D
Nucleo-pore® gDNA Fungal/Bacterial Mini Kit	50	NP-7006D

Product Warranty

RNASure® Blood Mini/Midi Kit components are intended for research purposes only. They are suitable for *in vitro* uses only. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, Genetix will replace it free of charge or refund the purchase price. Genetix reserve the right to change, alter, or modify any product to enhance its performance and design. It is the responsibility of the user to verify the use of the RNASure® Blood Mini/Midi Kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism. No claim or representation is intended for its use to identify any specific organism or for clinical or therapeutic use.

Genetix does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product.

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