
DNBSEQ rRNA & Globin Depletion Kit

- Cat. No.: 940-001810-00 (16 RXN)
940-001813-00 (96 RXN)
- Kit Version: V1.0

About the user manual

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Revision history

Manual Rev	Kit version	Date	Description
1.0	V1.0	Dec. 2023	Initial release

 Use the latest version of the manual and use it with the corresponding kit.

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1 Product overview

1.1 Introduction

DNBSEQ rRNA & Globin Depletion Kit depletes rRNA (including cytoplasmic 5 S rRNA, 5.8 S rRNA, 18 S rRNA, 28 S rRNA, mitochondrial ribosomal RNA 12 S rRNA, 16 S rRNA, and 45 S pre-ribosomal RNA) and Globin mRNA (including HBA1/2, HBB, HBD, HBG1/2, HBM, HBE1, HBQ1, HBZ) from Human blood total RNA in 10 ng-1 µg. The resulting mRNA and other non-coding RNA are suitable for downstream RNA analysis applications. This kit is compatible with both intact and degraded RNA. The library prepared by using this kit with RNA Library Prep Kit (e.g., DNBSEQ Fast RNA Library Prep Kit) is suitable for RNA quantitation, transcriptome or non-coding RNA research applications.





1.2 Intended use

This kit is applicable for total RNA samples from human blood.

1.3 Components

DNBSEQ rRNA & Globin Depletion Kit comes in two specifications: 16 RXN and 96 RXN. For component details, refer to the following table.

Table 1 DNBSEQ rRNA & Globin Depletion Kit (16 RXN) (Cat. No.: 940-001810-00)

Item & Cat. No.	Component	Cap color	Spec & Quantity
DNBSEQ rRNA & Globin Depletion Kit Cat. No.: 940-001810-00	rRNA Probe Mix	 White	32 µL/tube × 1
	Hybridization Buffer	 White	80 µL/tube × 1
	RNase H Buffer	 Orange	48 µL/tube × 1
	RNase H	 Orange	32 µL/tube × 1












Item & Cat. No.	Component	Cap color	Spec & Quantity
	DNase I Buffer	 Blue	280 µL/tube × 1
	DNase I	 Blue	40 µL/tube × 1
	Human Globin Probe Mix	 Purple	8 µL/tube × 1

Table 2 DNBSEQ rRNA & Globin Depletion Kit (96 RXN) (Cat. No.: 940-001813-00)

Item & Cat. No.	Component	Cap color	Spec & Quantity
DNBSEQ rRNA & Globin Depletion Kit Cat. No.: 940-001813-00	rRNA Probe Mix	 White	192 µL/tube × 1
	Hybridization Buffer	 White	480 µL/tube × 1
	RNase H Buffer	 Orange	288 µL/tube × 1
	RNase H	 Orange	192 µL/tube × 1
	DNase I Buffer	 Blue	840 µL/tube × 2
	DNase I	 Blue	240 µL/tube × 1
	Human Globin Probe Mix	 Purple	48 µL/tube × 1

1.4 Storage and transportation

DNBSEQ rRNA & Globin Depletion Kit

- Storage temperature: -25 °C to -15 °C
- Transportation temperature: -80 °C to -15 °C
 -  Production date and expiration date: refer to the label.
 - For dry ice shipments, ensure that there is enough dry ice remaining after transportation.

- With proper transport, storage, and use, all components can maintain complete activity within their shelf life.

1.5 User-supplied materials

Before the experiment, prepare the following equipment and materials. The "optional" material is determined by the actual requirement. For example, if DNase I Digestion is used for eliminating contaminating DNA in RNA sample, the digested product needs to be quantified. Therefore, you need to prepare "Qubit Fluorometer". If the RNA sample after depletion with rRNA and Globin mRNA needs the band distribution detection, you need to prepare "Agilent 2100 Bioanalyzer" and reagents.

Table 3 Order information for CG products

Catalog number	Model	Name
940-001518-00	16 RXN	DNBSEQ Fast RNA Library Prep Set
940-001522-00	B, 96 RXN	DNBSEQ Fast RNA Library Prep Set
940-001836-00	C, 96 RXN	DNBSEQ Fast RNA Library Prep Set
940-001515-00	192 RXN	DNBSEQ Fast RNA Library Prep Set

Table 4 User-supplied equipment list

Equipment	Recommended brand
Vortex mixer	/
Desktop centrifuge	/
Pipettes	/
Thermocycler	Bio-Rad, or Thermal Cycler with thermal gradient capability
Magnetic rack DynaMag -2, or equivalent	Thermo Fisher Scientific, Cat. No.: 12321D
Qubit Fluorometer, or equivalent	Thermo Fisher, Cat. No.: Q33216
Agilent 2100 Bioanalyzer, or equivalent	Agilent Technologies , Cat. No.: G2939AA

Table 5 Recommended reagent/consumable list

Reagent/consumable	Recommended brand
Nuclease Free (NF) water	Ambion, Cat. No.: AM9937, or equivalent
RNase Zap	Ambion, Cat. No. AM9780


Reagent/consumable	Recommended brand
RNA cleanup beads, Agencourt RNAClean XP 40 mL Kit	Beckman Coulter, Cat. No.: A63987, or equivalent
DNase I (optional)	NEB, Cat. No.: M0303S
100% Ethanol (Analytical Grade)	/
Agilent RNA 6000 Pico Kit	Agilent Technologies, Cat. No.: 5067-1513
Qubit RNA HS Assay Kit	Invitrogen, Cat. No.: Q32852
RNase-free tips	/
1.5 mL RNase-free non-stick microcentrifuge tubes	Ambion, Cat. No.: AM12450
0.2 mL RNase-free PCR tubes or 96-well plate	Axygen, Cat. No.: PCR-02-C or Axygen, Cat. No.: PCR-96M2-HS-C
Qubit Assay Tubes or 0.5 mL Thin Wall PCR Tubes	Invitrogen or Axygen, or equivalent



1.6 Precautions and warnings

- This product is for research use only, not for use in vitro diagnosis. Read this manual carefully before use.
- Familiarize yourself with the precautions and operation methods of various instruments before performing the experiment.
- This manual aims to provide a standard protocol. Changes can be made for different applications, but changes must be tested prior to starting the protocol.
- Wear masks and gloves before preparing RNA samples. Spray and wipe the pipette, test tube rack, and bench with RNase Zap RNase Decontamination Solution.
- It is recommended that you use pipette tips with filters to prevent cross-contamination. Use a new tip each time for pipetting different solutions or samples.
- It is recommended that you use the thermocyclers with heated lids for reactions. Preheat the thermocyclers to reaction temperature before use. If the thermocycler does not allow for lid temperature adjustments, the preset lid temperature of 105 °C is sufficient.
- Aerosol contamination may cause inaccurate results. It is recommended that you prepare separate working areas in the laboratory for PCR reaction preparation, PCR reaction, and PCR product cleanup. Use designated equipment for each area and clean the area regularly to ensure a sterile working environment (use 0.5% Sodium Hypochlorite or 10% Bleach to clean the working area).
- Avoid skin and eyes contact with samples and reagents. Do not eat or drink the samples and reagents. In case of contact with skin and eyes, rinse immediately with plenty of water and seek medical advice.
- Conform to the law and regulations when disposing of all samples and reagents.

- If you have questions, please contact Technical Support:
US-TechSupport@CompleteGenomics.com.

1.7 Workflow

Section	Workflow	Total time	Hands-on time
3.1	RNA/Probe hybridization	30 min	5 - 8 min
3.2	RNase H digestion	35 - 40 min	5 min
3.3	DNase I digestion	35 - 40 min	5 min
3.4	RNA cleanup	30 - 40 min	20 - 30 min
3.5	QC of rRNA depletion product 	15 - 60 min	10 - 20 min

-  • Total time: The theoretical use time of 8 reactions. The time will be extended if the number of reactions increases.
- Hands-on time: The total required hands-on time in the process.
-  Stop point.

2 Sample preparation

2.1 Sample requirements

2.1.1 Compatible sample type and input requirement

This kit depletes rRNA and Globin mRNA from human blood total RNA.


- For RNA sample with RIN (RNA Integrity Number) ≥ 7 , the recommended amount of RNA input is 10 ng - 1 μ g.
- For low-quality RNA sample (RIN < 7), the RNA input should not be less than 200 ng.

2.1.2 Total RNA quality requirements

- Use Agilent 2100 Bioanalyzer to perform quality for total RNA sample. RIN value should be ≥ 7 . If RIN < 7, increase RNA input (no more than 1 μ g) and the PCR cycles appropriately in the library preparation. If RIN is N/A, the sample is unqualified for library preparation.
- RNA purity: $OD_{260/280} = 1.8-2.0$, $OD_{260/230} \geq 2$.
The rRNA ratio will increase when $OD_{260/230} < 2$.
If $OD_{260/230} < 2$, total RNA is recommended to be purified with RNA cleanup beads before Chapter 3. The purification method can refer to “RNA cleanup” on page 11.
- To maximize the effect of rRNA depletion, ensure that there is no DNA contamination. If DNA contamination is found in the RNA sample (use agarose electrophoresis to detect), perform DNase I digestion to remove DNA. Refer to “DNase I digestion of RNA sample” on page 14 for instructions.
- If RNA sample is insufficient, you can try a lower amount of input for depletion. However, a lower amount of input might cause a decreased yield of PCR products and a lower comparison rate in the analysis result.

3 Library preparation protocol

3.1 RNA/probe hybridization

 **CAUTION** Do not shake or vortex the sample in the following procedures. Mix the sample by gently pipetting.

3.1.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

Table 6 Preparing the reagents

Reagent	Requirement
NF water	User-supplied. Place at room temperature (RT).
Hybridization Buffer	Mix thoroughly, centrifuge briefly, and place on ice.
rRNA Probe Mix	
Human Globin Probe Mix	

 Mix the Probe Mix by vortexing 5-6 times (3 sec each).

3.1.2 RNA/probe hybridization

1. According to the concentration of total RNA, transfer appropriate RNA sample (recommended 200 ng total RNA) to 0.2 mL PCR tube. Add NF water to make a total volume of 18 µL.

2. Take a new PCR tube, add probes according to the number of reactions and the table below, and vortex to mix the probe mixture thoroughly. It is recommended to prepare one more reaction.


 rRNA Probe Mix and Human Globin Probe Mix should be prepared into a probes mixture before adding to the sample. Adding them separately may lead to inaccurate addition amounts and affect the depletion effect.

Table 7 Probes mixture preparation (1 reaction)

Reagent	Volume per reaction
rRNA Probe Mix	1.5 μ L
Human Globin Probe Mix	0.5 μ L
Total	2 μ L

3. Individually add Probes mixture and Hybridization Buffer to RNA sample on ice as show in the table below.

 **CAUTION** Do not prepare a Probe Mix and Hybridization Buffer mixture.

Table 8 RNA/Probe hybridization reaction system

Reagent	Volume per reaction
Total RNA + NF water	18 μ L
Hybridization Buffer	5 μ L
Probes mixture (Step 2)	2 μ L
Total	25 μ L

4. Gently **pipette** the solution at least 15-20 times and centrifuge briefly. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.


 **CAUTION** After pipetting, make sure that there is no residue on the tip of the pipette; make sure that the number of pipetting times is sufficient to ensure that the hybridization reaction system is evenly mixed, otherwise the final depletion efficiency may be affected.

Table 9 RNA/Probe hybridization reaction conditions (Volume: 25 μ L)

Temperature	Time
105 $^{\circ}$ C Heated lid	On
95 $^{\circ}$ C	2 min
95 $^{\circ}$ C to 22 $^{\circ}$ C	0.1 $^{\circ}$ C /s
22 $^{\circ}$ C	5 min

5. The reaction takes approximately 20 min. After 22 $^{\circ}$ C for 5 min, immediately place the tube(s) on ice for 2 min. Centrifuge briefly and immediately proceed to the next step.

3.2 RNase H digestion

 **CAUTION** Do not shake or vortex the sample in the following procedures. Mix the sample by gently pipetting.

3.2.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

Table 10 Preparing the reagents

Reagent	Requirement
RNase H	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.
RNase H Buffer	Thaw on ice, mix by vortexing, centrifuge briefly, and place on ice.


3.2.2 RNase H digestion

1. According to the desired reaction number, prepare the RNase H digestion reaction mixture in a 0.2 mL PCR tube on ice. Mix it well by vortexing, centrifuge briefly, and place on ice.

Table 11 RNase H digestion reaction mixture

Reagent	Volume per reaction
RNase H	2 μ L
RNase H Buffer	3 μ L
Total	5 μ L

2. Add 5 μ L of RNase H digestion reaction mixture to each sample tube (from step 5 in 3.1.2). Gently **pipette** the solution at least 15-20 times, centrifuge briefly, and place on ice.

 **CAUTION** After pipetting, make sure that there is no residue on the tip of the pipette; make sure that the number of pipetting times is sufficient to ensure that the RNase H digestion system is evenly mixed, otherwise the final removal efficiency may be affected.

2. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 12 RNase H digestion reaction conditions (Volume: 30 µL)

Temperature	Time
45 °C Heated lid	On
37 °C	30 min
4 °C	hold

i If a heated lid cannot be set to 45 °C, set the temperature to the one closest to 45 °C.

4. After the reaction, centrifuge the tube(s) briefly and immediately proceed to the next step.

3.3 DNase I digestion



CAUTION Do not shake or vortex the sample in the following procedures. Mix the sample by gently pipetting.

3.3.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

Table 13 Preparing the reagents

Reagent	Requirement
DNase I	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.
DNase I Buffer	Thaw at RT, mix by vortexing, centrifuge briefly, and place on ice.

3.3.2 DNase I digestion

1. According to the desired reaction number, prepare the DNase I digestion reaction mixture in a 0.2 mL PCR tube on ice. Mix it well by vortexing, centrifuge briefly, and place on ice.

Table 14 DNase I digestion reaction mixture

Reagent	Volume per reaction
DNase I	2.5 µL
DNase I Buffer	17.5 µL
Total	20 µL

2. Add 20 μ L of DNase I digestion reaction mixture to each sample tube (from step 4 in 3.2.2). Gently **pipette** the mixture at least 15-20 times, centrifuge briefly, and place on ice.



CAUTION After pipetting, make sure that there is no residue on the tip of the pipette; make sure that the number of pipetting times is sufficient to ensure that the DNase I digestion system is evenly mixed, otherwise the final removal efficiency may be affected.



It is normal if bubbles appear during the mixing process. Centrifuge briefly and then flick to remove the bubbles.

3. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 15 DNase I digestion reaction conditions (Volume: 50 μ L)

Temperature	Time
45 °C Heated lid	On
37 °C	30 min
4°C	hold

4. After the reaction, centrifuge the tube(s) briefly and place on ice.

3.4 RNA cleanup



- Do not shake or vortex the sample in the following procedures. Mix the sample by gently pipetting.
- Use RNase-free non-stick tube in the RNA cleanup.
- Do not disturb or pipette the beads when adding reagents or transferring supernatant. If you accidentally disturb or pipette the beads, pipette the solution and beads back into the tube and restart the separation process.

3.4.1 Preparation

Reagent: For use with Agencourt RNAClean XP 40 mL Kit (user-supplied).

Table 16 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied. Freshly prepared with NF water .
NF water	User-supplied. Place at RT.
RNA Clean Beads	Allow 30 min to equilibrate to RT before use. Mix thoroughly by vortexing before each use.

3.4.2 RNA cleanup

- i** Transfer all liquid to a new RNase-free 1.5 mL tube (one tube per reaction) in advance if using a 1.5 mL centrifuge tube and a corresponding magnetic rack for purification.
1. Mix the RNA Clean Beads thoroughly. Add 75 μ L of RNA Clean Beads to each RNA sample tube (from step 3 in section 3.3.2). Gently **pipette** at least 10 times until all beads are suspended. Ensure that all of the solution and beads in the tip are transferred into the tube after mixing.
 2. Incubate the sample(s) at room temperature for 5 min.
 3. Centrifuge the sample tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Carefully remove and discard the supernatant. Do not disturb the beads that contain the RNA.
 4. While keeping the tube(s) on the magnetic rack, add 200 μ L of 80% ethanol to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.
 5. Repeat step 4. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
 6. Keep the tube(s) on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. There should be no visible cracking on the surface of the beads.

i Over-drying the beads will result in reduced yield.
 7. Remove the tube(s) from the magnetic rack and add an appropriate amount of NF water to elute the RNA. Gently **pipette** the liquid at least 10 times until all beads are suspended.

i In step 7, Elute the RNA sample from the beads, elute the RNA by adding an appropriate volume of NF water according to the following application.

For example, if you use MGIEasy Fast RNA Library Prep Kit, you need to add 12 μ L NF water to elute the RNA, and transfer 10 μ L of supernatant at step 9 to proceed to RNA fragmentation reaction.
 8. Incubate the sample(s) at room temperature for 5 min.
 9. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid is clear. Transfer the supernatant to a new RNase-free PCR tube.

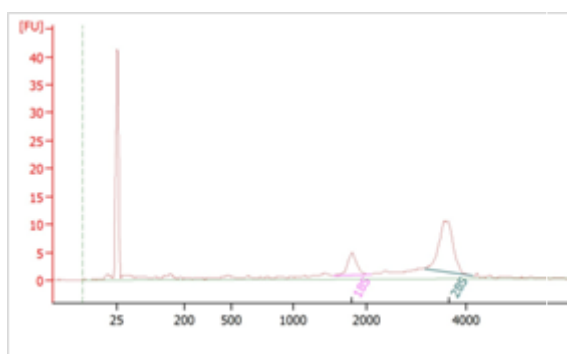
|| Stop point

 - Cleanup sample can be placed on ice for NGS library preparation or other application.
 - The product can be stored at -20 $^{\circ}$ C overnight or at -80 $^{\circ}$ C for no more than one week (we recommend that you proceed immediately to the next reaction).

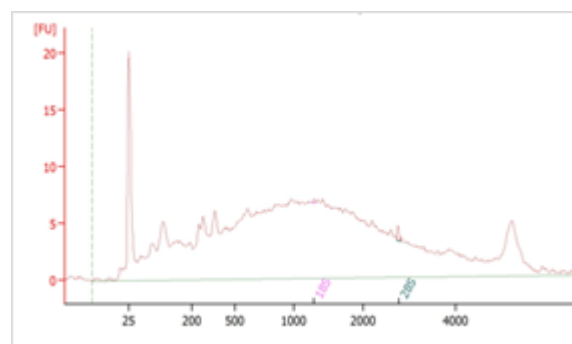
3.5 QC of depleted RNA

After the cleanup of the rRNA and Globin mRNA depleted RNA sample, perform the quality control following the instructions:

- i** This quality control is optional. If depleted RNA sample is proceeded by the NGS library construction, it is recommended that you skip this step.
- Take 1 μL of the cleanup sample for quality control using the Agilent RNA 6000 Pico chip (Refer to the Agilent RNA 6000 Pico chip manual for the instructions) and Agilent 2100 Bioanalyzer. In Figure 1A, two significant 18 S and 28 S rRNA peaks are present in the Agilent 2100 analysis result of the total RNA sample without depletion. In Figure 1B, no 18 S and 28 S rRNA peaks are present in the Agilent 2100 analysis result of the rRNA and Globin mRNA depleted RNA sample.



A



B

Figure 1 Agilent RNA 6000 Pico assay result of purified rRNA depleted RNA

A. The Agilent 2100 analysis result of the Human blood total RNA sample without rRNA depletion;


B. The Agilent 2100 analysis result of the rRNA depleted Human blood total RNA sample.

4 Appendix

4.1 DNase I digestion of RNA sample

4.1.1 DNase I digestion of RNA sample

If there is DNA contamination in RNA sample, you need to perform DNase I digestion. DNase I digestion causes a certain loss of RNA sample, the amount of total RNA used in this step needs to be increased by 20% to 30% more than the expected input (the amount of RNA input for rRNA depletion required in this kit). For example, for rRNA depletion, if the required input amount of RNA is 200 ng, the total RNA input should be 250-286 ng for the DNase I digestion.

 **CAUTION** Do not shake or vortex the sample in the following procedures. Mix the sample by gently pipetting.

4.1.1.1 Preparation

Mix the reagents before using and store the remaining reagents immediately after use.

Table 17 Preparing the reagents

Reagent	Requirement
NF water	User-supplied. Place at RT.
DNase I	Flick and/or invert the tube gently, centrifuge briefly, and place on ice.
DNase I Buffer	Thaw at RT, mix by vortexing, centrifuge briefly, and place on ice.

4.1.1.2 DNase I digestion

1. Transfer an appropriate amount of RNA sample to a RNase free 0.2 mL PCR tube. Add NF water to make a final volume of 42.5 μ L.
2. According to the desired reaction number, dilute the DNase I Buffer in a 1:10 dilution with NF Water (5 μ L of 10x DNase I Buffer per reaction).

3. According to the desired reaction number, prepare the DNase I digestion reaction mixture on ice.

Table 18 DNase I digestion reaction mixture

Reagent	Volume per reaction
DNase I	2.5 μ L
10x DNase I Buffer	5 μ L
Total	7.5 μ L


4. Add 7.5 μ L of DNase I digestion reaction mixture to each sample tube. Gently **pipette** the mixture at least 10 times and centrifuge briefly.
5. Place the PCR tube(s) into the thermocycler. Run the program with the following conditions.

Table 19 DNase I digestion reaction conditions (Volume: 50 μ L)

Temperature	Time
45 °C Heated lid	On
37 °C	20 min
4°C	hold

6. After the reaction, centrifuge the tube(s) briefly and place on ice.

4.1.2 Cleanup of RNA sample

-  Do not shake or vortex the sample in the following procedures. Mix the sample by gently pipetting.
- Use RNase-free non-stick tube in the RNA cleanup.
- Do not touch or pipette the beads when adding reagents or transferring supernatant. If accidentally pipetting the beads, pipette all of the solution and beads back into the tube and restart the separation process.

4.1.2.1 Preparation

Reagent: For use with Agencourt RNAClean XP 40 mL Kit (user-supplied).

Table 20 Preparing the reagents

Reagent	Requirement
80% ethanol	User-supplied. Freshly prepared with NF water .
NF water	User-supplied. Place at RT.

Reagent	Requirement
RNA Cleanup Beads	User-supplied. Take out 30 min in advance to equilibrate to RT. Mix thoroughly by vortexing before each use.

4.1.2.2 Cleanup of RNA sample

i Transfer all liquid to a new RNase-free 1.5 mL tube (one tube per reaction) in advance if using a 1.5 mL centrifuge tube and a corresponding magnetic rack for purification.

1. Mix the RNA Cleanup Beads thoroughly. Add 90 μ L of RNA Cleanup Beads to each RNA sample tube (from 4.1.1.2 step 6). Gently **pipette** at least 10 times until all beads are suspended. Ensure all of the solution and beads in the tip are transferred into the tube after mixing.
2. Incubate the sample(s) at room temperature for 5 min.
3. Centrifuge the sample tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid becomes clear. Carefully remove and discard the supernatant. Be careful not to disturb the beads that contain the RNA.
4. While keeping the tube(s) on the magnetic rack, add 200 μ L of 80% ethanol to each tube to wash the beads and tube wall. Wait for 30 sec. Carefully remove and discard the supernatant.
5. Repeat step 4. Try to remove all liquid from the tube. If some liquid remains on the tube wall, centrifuge the tube briefly and place it on the magnetic rack for separation. Remove all liquid by using a low-volume pipette.
6. Keep the centrifuge tube(s) on the magnetic rack. Open the tube cap and air-dry the beads at room temperature until no wetness or glossiness is visible on the beads' surface. Cracking on the surface of the beads should not be observed.

i Over-drying the beads will result in reduced yield.

7. Remove the centrifuge tube(s) from the magnetic rack, and add appropriate amount of NF water to elute the RNA. Gently **pipette** the liquid at least 10 times until all beads are suspended.

i ■ If the purified RNA sample needs to be quantitated, elute the RNA with 21 μ L of NF water. Transfer 19 μ L of supernatant to a new RNase free PCR tube and take 1 μ L of product for quantitation using Qubit RNA HS Assay Kit.

- If the quantitation is not performed, elute the RNA with 20 μ L of NF water and transfer 18 μ L of supernatant to a new RNase free PCR tube for RNA/Probe hybridization.

8. Incubate the sample(s) at room temperature for 5 min.
9. Centrifuge the tube(s) briefly and place on the magnetic rack for 2 to 5 min until the liquid becomes clear. Transfer the supernatant to a new RNase-free PCR tube and proceed to the "RNA/probe hybridization" on page 7.

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