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MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit

(Compatible with PAXgene® Blood RNA Tubes)

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Note: For safety and biohazard guidelines, refer to the "Safety" section in the *MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit User Guide* (Part no. 4452006). For every chemical, read the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This quick reference includes step-by-step instructions for RNA isolation from PAXgene® Blood RNA Tube-stabilized blood samples using the MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit (Part no. 4451894). For background information, reagent and equipment preparation, troubleshooting, and supplemental procedures, refer to the *MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit User Guide*.

Prepare the PAXgene®-stabilized blood samples

Centrifuge the samples and wash the crude RNA pellets

1. If the blood samples are frozen:
   a. Thaw the samples in the PAXgene® Blood RNA Tubes for at least 2 hours at room temperature (18 to 25°C).
   b. Gently invert the thawed blood samples 10 times.
2. Centrifuge the PAXgene® tubes at 3000 × *g* for 10 minutes to pellet the crude RNA.
3. Carefully pour the supernatant from each tube.
4. To each tube, add 5 mL of nuclease-free water, then securely re-cap the tubes.
5. Vortex the tubes at vigorous speed until the crude RNA pellets are fully resuspended.
6. Centrifuge the tubes at 3000 × *g* for 10 minutes to re-pellet the crude RNA .
7. Carefully pour the supernatant from each tube.
8. Drain residual supernatant by inverting the tubes on absorbent paper for 2 minutes.
9. Using clean, absorbent paper, blot any remaining liquid from the tube rims.

Resuspend the washed crude RNA pellets and digest with protease

1. Just before use, prepare the resuspension mixture.
   a. Per the table below, calculate the total volume required for each component: *volume for 1 sample × the total number of samples*

      Include 5% excess volume in your calculations to compensate for the loss that occurs during pipetting.

Component	Volume
Resuspension Solution†	174 µL
Proteinase†	6 µL
Total volume required for 1 sample	**180 µL**

† Compatible with PAXgene® Blood RNA Tubes, not compatible with Tempus® Blood RNA Tubes.

   b. Add the components to a microcentrifuge tube, then mix well by vortexing at moderate speed.
   c. Briefly (2 to 3 seconds) centrifuge to collect the resuspension mixture at the tube bottom.
   d. Place the resuspension mixture on ice until needed.
2. To each PAXgene® tube, add 180 µL of the prepared resuspension mixture, then securely re-cap the tubes.
3. Vortex the tubes at vigorous speed until the crude RNA pellets are fully resuspended.
4. For *each* PAXgene® tube, transfer the total volume of resuspended crude RNA pellet to one 2.0 mL Tube (provided with the kit), then securely cap and label each 2.0 mL Tube with the sample identification.
5. Place the 2.0 mL Tubes in the pre-heated thermomixer, then mix at 1000 rpm for 10 minutes at 55°C to digest.

IMPORTANT! It is critical that you perform the protease digestion at the specified temperature and mixing speed. Otherwise, RNA yields and quality may decrease significantly.

6. Centrifuge the 2.0 mL Tubes at 16,000 × *g* for 3 minutes to clarify the digested samples.

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7. For *each* 2.0 mL Tube: Without disturbing the debris pellet, transfer the total volume (~180 to 200 µL) of RNA-rich supernatant to:
   - One 1.5-mL microfuge tube; securely cap and label each tube with the sample identification

     OR
   - One well of the 96-well Processing Plate

   Note: Small amounts of the debris pellet may be aspirated with the RNA-rich supernatant. This will not interfere with RNA purification.

STOPPING POINT You can store the RNA-rich supernatant at room temperature (18 to 25°C) for up to 4 hours. If you are using the 96-well Processing Plate, place a cover over all wells that contain the RNA-rich supernatant.

Purify the RNA

RNA purification procedure guidelines

- Ensure that Wash Solutions 1 and 2 have been prepared by addition of isopropanol or ethanol, as indicated on the bottle.
- Perform the RNA purification procedure at room temperature (18 to 25°C), unless otherwise stated.
- When aspirating, be careful not to dislodge the RNA Binding Beads from the magnet.
- The capture time of the RNA Binding Beads depends on the magnetic stand that you use.
- When capturing the RNA Binding Beads on the magnetic stand, you can remove the supernatant after the solution becomes clear and the beads form a pellet at the magnet.
- When performing a tube-based purification, securely cap the tubes before shaking them on the vortex adaptor.
- When performing a tube-based purification, briefly (2 to 3 seconds) centrifuge the sample each time after shaking in the vortex adapter to collect the contents at the tube bottom.

Bind the RNA to the RNA Binding Beads, then magnetically capture the beads

1. Vortex the RNA Binding Beads at vigorous speed until fully resuspended.
2. To each microfuge tube or well (containing RNA-rich supernatant), add 20 µL of the vortexed RNA Binding Beads, then mix at moderate speed for 3 minutes:
   - Shake the tubes on a vortex adaptor (settings 4 to 5).
   - Shake the plate on an orbital shaker (settings 6 to 7).
3. To each microfuge tube or well, add 200 µL of 100% isopropanol, then mix at moderate speed for 5 minutes:
   - Shake the tubes on a vortex adaptor (settings 4 to 5).
   - Shake the plate on an orbital shaker (settings 6 to 7).

   Note: After you add the 100% isopropanol, the RNA Binding Beads may clump. This will not interfere with RNA purification.
4. Use a magnetic stand to capture the RNA Binding Beads. The capture time is ~1 to 3 minutes.
5. Carefully aspirate and discard all supernatant without disturbing the beads.

   Note: The supernatant may be dark and opaque during the first binding. When you remove the supernatant, note where the magnet contacts the wells of the tubes or plate so that you can angle the pipette tips away from the magnetic beads.
6. Remove the microfuge tubes or plate from the magnetic stand.

   IMPORTANT! It is critical that you remove the tubes or plate from the magnetic stand before you perform the next step.

Wash once with Wash Solution 1

1. To each microfuge tube or well, add 150 µL of prepared Wash Solution 1, then mix at moderate speed for 1 minute:
   - Shake the tubes on a vortex adaptor (settings 4 to 5).
   - Shake the plate on an orbital shaker (settings 6 to 7).
2. Use a magnetic stand to capture the RNA Binding Beads. The capture time is ~1 to 2 minutes.
3. Carefully aspirate and discard all supernatant without disturbing the beads.
4. Remove the microfuge tubes or plate from the magnetic stand.

   IMPORTANT! It is critical that you remove the tubes or plate from the magnetic stand before the next step.

Wash once with Wash Solution 2

1. To each microfuge tube or well, add 150 µL of prepared Wash Solution 2, then mix at moderate speed for 1 minute:
   - Shake the tubes on a vortex adaptor (settings 4 to 5).
   - Shake the plate on an orbital shaker (settings 6 to 7).
2. Use a magnetic stand to capture the RNA Binding Beads. The capture time is ~1 to 2 minutes.
3. Carefully aspirate and discard all supernatant without disturbing the beads.
4. Remove the microfuge tubes or plate from the magnetic stand.

   IMPORTANT! It is critical that you remove the tubes or plate from the magnetic stand before the next step.

Treat with TURBO™ DNase, then rebind the RNA

1. Just before use, prepare the TURBO™ DNase mixture:
   a. Per the table below, calculate the total volume required for each component: *volume for 1 sample × the total number of samples*

      Include 5% excess volume in your calculations to compensate for the loss that occurs during pipetting.

Component	Volume
Nuclease-free water	35 µL
10× TURBO™ DNase Buffer	5 µL
TURBO™ DNase	10 µL
Total volume required for 1 sample	**50 µL**

   b. Add the components to a microcentrifuge tube, then mix well by vortexing at gentle speed.
   c. Briefly (2 to 3 seconds) centrifuge to collect the TURBO DNase mixture at the tube bottom.
   d. Place the TURBO™ DNase mixture on ice until needed.
2. To each microfuge tube or well, add 50 µL of the prepared TURBO™ DNase mixture, then mix at vigorous speed for 10 minutes:
   - Shake the tubes on a vortex adaptor (settings 6 to 7).
   - Shake the plate on an orbital shaker (settings 8 to 9).
3. To each microfuge tube or well, add:
   - 50 µL of RNA Rebinding Buffer
   - 100 µL of 100% isopropanol

   Note: The RNA Binding Beads should not clump after you add the 100% isopropanol.
4. Mix at moderate speed for 3 minutes:
   - Shake the tubes on a vortex adaptor (settings 4 to 5).
   - Shake the plate on an orbital shaker (settings 6 to 7).
5. Use a magnetic stand to capture the RNA Binding Beads. The capture time is ~1 to 2 minutes.
6. Carefully aspirate and discard all supernatant without disturbing the beads.
7. Remove the microfuge tubes or plate from the magnetic stand.

   IMPORTANT! It is critical that you remove the tubes or plate from the magnetic stand before the next step.

Wash twice with Wash Solution 2

1. To each microfuge tube or well, add 150 µL of prepared Wash Solution 2, then mix at moderate speed for 1 minute:
   - Shake the tubes on a vortex adaptor (settings 4 to 5).
   - Shake the plate on an orbital shaker (settings 6 to 7).
2. Use a magnetic stand to capture the RNA Binding Beads. The capture time is ~1 to 2 minutes.
3. Carefully aspirate and discard all supernatant without disturbing the beads.
4. Remove the microfuge tubes or plate from the magnetic stand.

   IMPORTANT! It is critical that you remove the tubes or plate from the magnetic stand before the next step.
5. Repeat steps 1 to 4 above once.

Dry the beads

To dry the beads in microfuge tubes:

1. Open the tubes, then invert the tubes on absorbent paper for 2 minutes at room temperature.
2. Inspect the tubes. If there is residual Wash Solution 2:
   a. Use a fine-tipped pipette to remove the supernatant, being careful not to disturb the beads. Discard the supernatant.
   b. Leaving the tubes open, invert for 1 minute more at room temperature.

To dry the beads in a 96-well Processing Plate:

1. Shake the plate on an orbital shaker at vigorous speed (settings 8 to 9) for 2 minutes at room temperature.
2. Inspect the plate. If there is residual Wash Solution 2, shake the plate at vigorous speed (settings 8 to 9) for 2 minutes more at room temperature.

Elute the RNA

1. To each microfuge tube or well, add 80 µL of Elution Buffer, then mix at vigorous speed for 4 minutes:
   - Shake the tubes on a vortex adaptor (settings 6 to 7).
   - Shake the plate on an orbital shaker (settings 8 to 9).

   Note: You can use 20 to 80 µL of Elution Buffer, depending on the final concentration you want to obtain.
2. Use a magnetic stand to capture the RNA Binding Beads. The capture time is ~1 to 3 minutes.
3. Being careful not to disturb the beads, transfer the supernatant to a nuclease-free container that is appropriate for your application. **Do not discard the supernatant; the purified RNA is in the supernatant.**

STOPPING POINT Store the purified RNA on ice for immediate use, at –20°C for up to 6 months, or at –80°C for long-term storage.

Kit contents and storage

Kit part no.	Kit name	Kit contents			Storage conditions
		Box	Component	Amount	
4451894	MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit (Compatible with PAXgene® Blood RNA Tubes)	1	Resuspension Solution (Compatible with PAXgene® Blood RNA Tubes)	18 mL	Room temperature
			Wash Solution 1 Concentrate†	16 mL	
			Wash Solution 2 Concentrate‡	2 × 30 mL	
			RNA Rebinding Buffer	10 mL	
			Elution Buffer	10 mL	
			Processing Plate (96-well)	1 plate	
			2.0 mL Tubes	100 tubes	
			RNA Binding Beads	2 mL	2 to 8°C Do not freeze.
		2	Proteinase (Compatible with PAXgene® Blood RNA Tubes)	0.6 mL	–15 to –25°C
			10× TURBO™ DNase Buffer	1 mL	
			TURBO™ DNase	1 mL	

† Before using the Wash Solution 1 Concentrate, add the correct volume of isopropanol (user-supplied), as indicated on the bottle.
‡ Before using the Wash Solution 2 Concentrate, add the correct volume of ethanol (user-supplied), as indicated on the bottle.

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

NOTICE TO PURCHASER: PLEASE REFER TO THE MAGMAX™ FOR STABILIZED BLOOD TUBES RNA ISOLATION KIT (COMPATIBLE WITH PAXGENE® BLOOD RNA TUBES) USER GUIDE (PART NO. 4452006) FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

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