MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit

(Compatible with Tempus® Blood RNA Tubes)

Note: For safety and biohazard guidelines, refer to the "Safety" section in the *MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit (Compatible with Tempus® Blood RNA Tubes) Protocol* (PN 4452007). For every chemical, read the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This quick reference card covers:

Prepare the Tempus®-stabilized blood samples

Dilute the samples with Tempus® 1× PBS

IMPORTANT! Keep the samples on ice as much as possible. Otherwise, RNA yields may decrease significantly.

For *each* blood sample being processed:

1. If the sample is frozen, thaw the sample in the Tempus® Blood RNA Tube for 10 to 30 minutes on ice.
2. Label a clean 50-mL conical tube with the sample identification.
3. Pour the entire contents of the Tempus tube into the conical tube.
4. Add 3 mL of Tempus® 1× PBS to the conical tube to bring the total volume to 12 mL.

   IMPORTANT! If the initial blood sample was less than 3 mL, make up the difference by adding enough Tempus 1× PBS to bring the total volume to 12 mL. Otherwise, RNA yields decrease significantly.
5. Securely cap the conical tube, then vortex at vigorous speed for 30 seconds to ensure proper mixing of the contents.

   IMPORTANT! To prevent the tube from leaking and spraying the sample during vortexing, make sure that the tube is securely capped.

   Note: Frothing of the sample after vortexing is normal.

Centrifuge the samples and wash the crude RNA pellets

1. Centrifuge the conical tubes at 5000 × *g* for 15 minutes at 4 °C to pellet the crude RNA.

   Note: The crude RNA pellet in each tube is transparent and invisible.

   IMPORTANT! Handle each tube carefully so that you do not dislodge the crude RNA pellet from the tube bottom.
2. Carefully pour the supernatant from each tube.
3. Add the Tempus® Pre-Digestion Wash:

   IMPORTANT! Before using the Tempus Pre-Digestion Wash, mix well by gently inverting the bottle 3 to 4 times to ensure it is a homogenous solution.

   a. To each conical tube, add 4 mL of the Tempus Pre-Digestion Wash, then securely re-cap the conical tubes.

   b. Vortex the conical tubes at moderate speed for 5 seconds.

   Note: The vortex speed setting should gently draw the Tempus Pre-Digestion Wash three-quarters of the way up the inside of the conical tube.
4. Centrifuge the conical tubes at 5000 × *g* for 10 minutes at 4 °C to re-pellet the crude RNA.
5. Carefully pour the supernatant from each tube, being careful not to dislodge the crude RNA pellet.
6. Drain residual supernatant by inverting the tubes on absorbent paper for 2 minutes.
7. Using clean, absorbent paper, blot any remaining liquid from the tube rims.
8. Place the conical tubes on ice until needed.

Resuspend the washed crude RNA pellets and digest with protease and TURBO™ DNase

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
Tempus® Resuspension Solution	117.5 µL
Tempus® Proteinase	2.5 µL
Total volume required for 1 sample	**120 µL**

   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 conical tube, add 120 µL of the prepared resuspension mixture, then securely re-cap the tubes.
3. Vortex the tubes at gentle speed for 5 to 10 seconds to fully resuspend the crude RNA pellets.
4. For *each* conical tube, transfer the total volume of resuspended crude RNA pellet to:
   - One 1.5-mL microfuge tub; securely cap and label each tube with the sample identification

     OR
   - One well of the 96-well Processing Plate

STOPPING POINT You can store the resuspended crude RNA pellets on ice for up to 4 hours. If you are using the 96-well Processing Plate, place a cover over all wells that contain a resuspended pellet. Remove the resuspended pellets from the ice and remove the cover just before proceeding to step 5 below.

5. To each microfuge tube or to each well (containing a resuspended crude RNA pellet), add 10 µL of TURBO™ DNase, then mix at vigorous speed for 10 minutes:
   - Shake the microfuge tubes on a vortex adaptor (settings 6 to 7).
   - Shake the plate on an orbital shaker (settings 8 to 9).

Purify the RNA

RNA purification procedure guidelines

- 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 an RNA sample), add:
   - 50 µL of Binding Solution Concentrate
   - 20 µL of the vortexed RNA Binding Beads
3. 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).
4. To each microfuge tube or well, add 200 µL of 100% isopropanol, 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).
5. Use a magnetic stand to capture the RNA Binding Beads. The capture time is ~1 to 3 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 you perform the next step.

Wash twice 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.
5. Repeat steps 1 to 4 above once.

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.

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 TEMPUS® BLOOD RNA TUBES) PROTOCOL FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

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