# Tempus™ Spin RNA Isolation Reagent Kit

## Product Insert

Product: P/N 4378926
Insert: P/N 4382075 REV B
Printed in USA

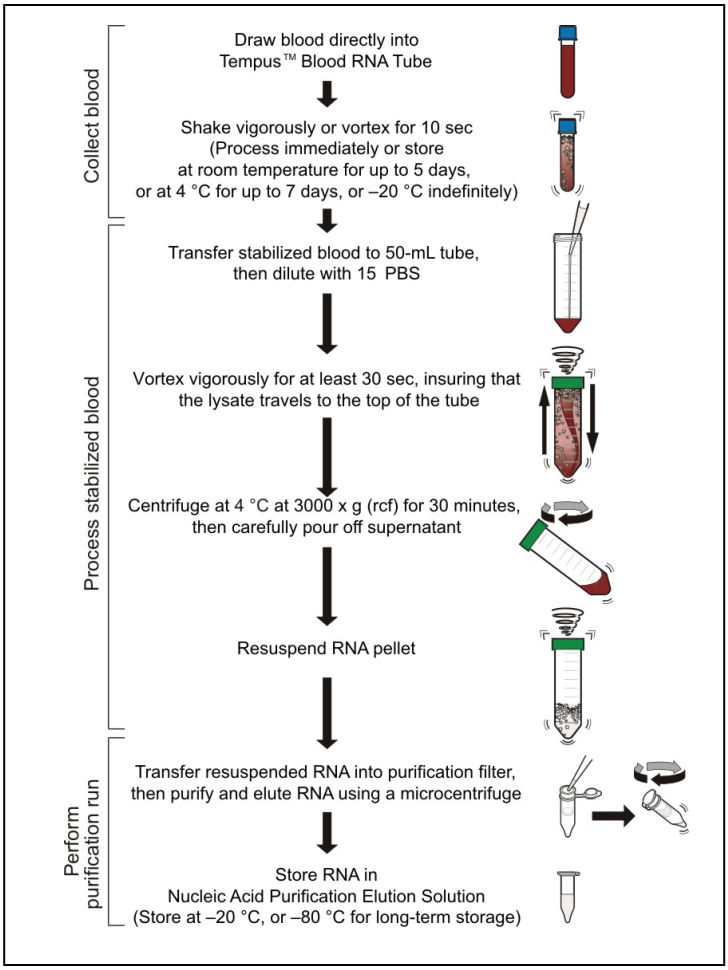
For safety and biohazard guidelines, refer to the "Safety" section in the *Tempus™ Blood RNA Tube* and *Tempus™ Spin RNA Isolation Kit Protocol* (PN 4379232). For all chemicals in **bold red** type below, read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Kit Content:

1X PBS Buffer
**Resuspension Solution**
**Wash Solution 1**
**Wash Solution 2**
**Elution Solution**
Spin Columns (50 each)

## Procedure Overview

The following diagram provides an overview of the procedure for using the Tempus™ Spin RNA Isolation Kit to isolate RNA from human whole blood collected in a Tempus™ Blood RNA Tube.



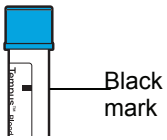

## Collecting and Storing Blood in Tempus™ Blood RNA Tubes

### Collecting Blood

1. Draw 3 mL of blood directly into the **Tempus™ Blood RNA Tube**, following your laboratory's standard procedures for drawing blood from individuals into blood collection tubes containing liquid reagents. Observe the appropriate safety practices when collecting blood.

   **Note:** If you are using the Greiner Vacuette® Safety Blood Collection Set, refer to the Vacuette Web site (**www.vacuette.com**) for additional information.

   **Note:** Filling up the tube to the black mark on the tube label indicates the collection of approximately 3 mL of blood.




2. Immediately after the Tempus tube is filled, stabilize the blood by shaking vigorously or vortexing the contents for 10 seconds to ensure that the Applied Biosystems Stabilizing Reagent makes uniform contact with the sample.

   **IMPORTANT!** Failure to mix the stabilizing reagent with the blood leads to inadequate stabilization of the gene expression profile and the formation of microclots that can potentially clog the purification filter.

## Storing and Transporting Blood in Tempus™ Blood RNA Tubes

Applied Biosystems recommends that you store or ship Tempus tubes containing stabilized samples in the following order of preference:

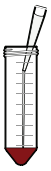
| Storage / Shipping Options | Temperature Requirement ( °C) |
|---|---|
| Store or ship refrigerated within 7 days or less.<br><br>(Recommended) | 4 |
| Store or ship on dry ice.<br><br>**IMPORTANT!** Avoid direct contact of sample with dry ice! | –20 to –80 |
| Store or ship at room temperature within 5 days or less. | 18 to 25 |

## Processing Stabilized Blood Before Purification

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

1. If the sample is frozen, thaw the sample in the Tempus tube at room temperature (18 to 25 °C).

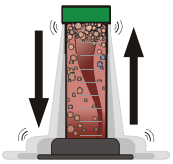
2. Remove the cap from the Tempus tube, then pour the contents of the tube into a clean 50-mL tube (such as a 50-mL Ambion conical tube).



3. Pipet 3 mL of **1X Phosphate-Buffered Saline** (PBS; Ca2+/Mg2+-free) into the 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 1X PBS to bring the total volume to 12 mL.

4. Replace the cap on the tube, then vortex the tube vigorously (at maximum vortex speed) for 30 seconds to ensure proper mixing of contents.

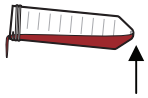


   **Note:** To prevent the tube from leaking and spraying the samples during vortexing, make sure the tube is capped properly.

   **IMPORTANT!** Vortex the diluted sample for at least 30 seconds; vortexing for less than 30 seconds may cause clogging of the purification consumable.

   **Note:** Frothing of the samples after vortexing is normal.

5. Centrifuge the tube at **4 °C** at **3000 x *g* (rcf)** for 30 minutes.

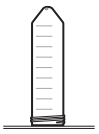


RNA pellet

6. Carefully pour off the supernatant.

   **Note:** The RNA pellet is transparent and invisible.

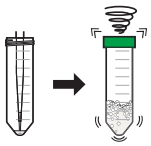
   **IMPORTANT!** Handle the tube carefully so that you do not shake the RNA pellet off the bottom of the tube.



7. Leave the tube inverted on absorbent paper for 1 to 2 minutes.

8. Blot the remaining drops of liquid off the rim of the tube with clean absorbent paper.

9. Pipet 400 µL of **RNA Purification Resuspension Solution** into the tube, then vortex briefly to resuspend the RNA pellet.



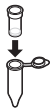
   **IMPORTANT!** To prevent washing any blood residue down the inside of the tube, insert the pipet tip into the tube and add the resuspension solution to the bottom of the tube

10. Keep resuspended sample on ice while preparing for the purification steps.

## Performing the Purification Run

**Note:** The RNA isolated in this procedure contains very low levels of genomic DNA (less than 0.05% by weight). If you are using the RNA with assays for low-expressing genes, you may want to perform an optional DNase treatment to further reduce the trace amounts of DNA that might interfere with signal detection and mask signals.

1. Label the RNA purification filter, and then insert the filter into a collection tube.

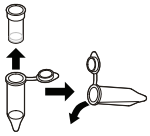


2. Pre-wet the filtration membrane by pipeting **RNA Purification Wash Solution 1** into the purification filter.

| Wash Solution 1 | Time | Centrifuge |
|---|---|---|
| 100 µL | — | — |

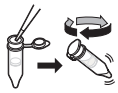
3. Pipet the resuspended RNA into the purification filter, and then centrifuge.

| Resuspended RNA | Time | Centrifuge |
|---|---|---|
| ~400 µL | 30 sec | 16,000 x *g* (rcf) |

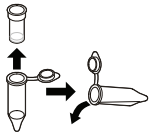


4. Remove the purification filter, discard the liquid waste collected in the waste tube, and then re-insert the purification filter into waste tube.

5. Pipet RNA Purification Wash Solution 1 into the purification filter, and then centrifuge.

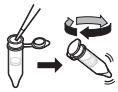


| Wash Solution 1 | Time | Centrifuge |
|---|---|---|
| 500 µL | 30 sec | 16,000 x *g* (rcf) |



6. Remove the purification filter, discard the liquid waste collected in the waste tube, and then re-insert the purification filter into waste tube.

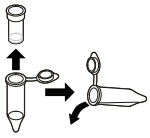
7. Pipet **RNA Purification Wash Solution 2** into the purification filter, and then centrifuge.



**IMPORTANT!** When a DNase treatment is required, extend the centrifuge time to 1 minute to remove all wash solutions and dry the membrane completely.

| Wash Solution 2 | Time | Centrifuge |
|---|---|---|
| 500 µL | 30 sec‡ | 16,000 x *g* (rcf) |

‡ 60 sec, if a DNase treatment is required



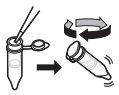
8. Remove the purification filter, discard the liquid waste collected in the waste tube, and then re-insert the purification filter into waste tube.

9. (Optional) Perform a DNase treatment:

   a. Pipet AbsoluteRNA Wash Solution (not provided) into the purification filter, and then incubate at room temperature.

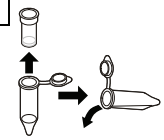
| AbsoluteRNA Wash Solution | Time | Centrifuge |
|---|---|---|
| 100 µL | 15 min | — |

   b. Pipet RNA Purification Wash Solution 2 into each purification filter, incubate, and then centrifuge.

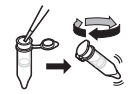


| Wash Solution 2 | Time | Centrifuge |
|---|---|---|
| 500 µL | 5 min | — |
| | 30 sec | 16,000 x (rcf) |

   c. Remove the purification filter, discard the liquid waste collected in the waste tube, and then re-insert the purification filter into waste tube.

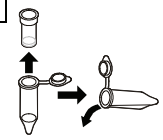


10. Pipet RNA Purification Wash Solution 2 into the purification filter, and then centrifuge.



| Wash Solution 2 | Time | Centrifuge |
|---|---|---|
| 500 µL | 30 sec | 16,000 x *g* (rcf) |

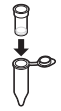
11. Remove the purification filter, discard the liquid waste collected in the waste tube, and then re-insert the purification filter into waste tube.



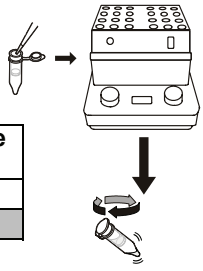
12. Centrifuge to dry the membrane.

| Solution | Time | Centrifuge |
|---|---|---|
| — | 30 sec | 16,000 x *g* (rcf) |

13. Transfer the purification filter to a new, labeled collection tube to collect the eluate.

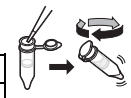


14. Pipet **Nucleic Acid Purification Elution Solution** into the purification filter, close the cap, incubate the entire tube, and then centrifuge.



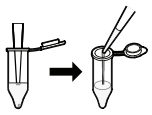
| Elution Solution | Time | Centrifuge | Incubate |
|---|---|---|---|
| 100 µL | 2 min | — | 70 °C |
| | 30 sec | 16,000 x *g* | — |

15. Pipet the collected RNA eluate back into the purification filter, and then centrifuge.



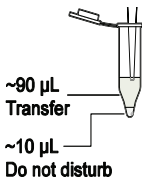
| RNA Eluate | Time | Centrifuge |
|---|---|---|
| ~100 µL | 2 min | Maximum (16,000 to 18,000 x *g*) |

16. Discard the purification filter, and then transfer the approximately 90 µL of the RNA eluate to a new, labeled collection tube.



**IMPORTANT!** When transferring the RNA eluate, carefully pipet the liquid out of the collection tube starting from the top of the liquid to ensure that the pelleted particulates are not disturbed.

17. Replace the cap on the new collection tube, and store the RNA at –20 °C for short-term storage, or –80 °C for long-term storage.



**For Research Use Only. Not for use in diagnostic procedures.**

# VENDOR INFORMATION,
# PRODUCT, CUSTOM INSERT LAYOUT
# 17 x 11 SHEET - BOTH SIDES PRINTED COLOR (4/4)

Insert P/N 4382075 REV B

## GUIDELINES, CUSTOM INSERT LAYOUT, CUSTOM FOLD INSTRUCTIONS

This page is folding instructions which is NOT and MUST NOT BE PRINTED as part of this insert.

1 Print insert in color.
2 Specification is intended for 17 x 11 sheet, only.
3 Material: 50# Offset (White)
4 Dimensions: Full Sheet (17" x 11") folded size = 4.25" x 2.75 " +/-.125"
5 Two sheet inserts to have pages 2 and 3 facing each other.
6 After final folding, upper left-hand corner of page 1 is to face out, with title and part number displayed.
7 Folded inserts to be packaged as follows:
- Bundled in groups of 10ea.
- Shrink-wrap 10ea groups for 100 inserts total.
- Label shrink-wrapped bundles [Part# & Rev (font large enough to be legible), Qty, Manufacturer, and Barcode Part #128C].
- Seal shrink-wrapped bundles in an appropriately sized corrugated shipper (not to exceed 25#, or have outside dimensions greater than 12" x 13 ½" x 24").
- Appropriately label shipper [Part# & Rev (font large enough to be legible at 8 feet), Description, P.O.#, Qty, Mfg Date, Manufacturer, Country of Origin, and Barcode Part # in 128C] on at least one end panel (12" x 13 ½" side).