Tempus™ Blood RNA Systems
New! Tempus™ Spin RNA Isolation Kit

A Complete System for Stabilizing and Isolating RNA from Whole Blood

Tempus™ Blood RNA Tubes

- Collect 3 mL of whole blood and immediately stabilize RNA transcripts at point of collection
- Lock global gene expression profiles for up to 5 days at room temperature and ≥ 7 days at 4° C
- Compatible with two RNA isolation formats: Spin Column or Vacuum Filtration

New! Tempus™ Spin RNA Isolation Kit

- Isolate high-quality RNA suitable for real-time PCR, microarray analysis, or other RNA analysis methods
- Easy workflow using standard laboratory centrifuges to isolate RNA in approximately 1 hour
- RNA purity with A260/280 ratio >1.9 and intact RNA with RIN profiles > 7.0
- Isolate 6 to 25 µg of RNA from 3 mL blood
- No separate RBC lysis or proteinase K treatment required
- Highly stable RNA virtually free of RNase activity, contaminating gDNA, or RT-PCR inhibitors
- No specialized equipment necessary

Introduction

Gene expression profiling and analysis from whole blood is becoming an increasingly important life science tool. Accurately measuring the molecular profile of blood-based RNA transcripts is a critical need in areas of research such as disease association studies, biomarker discovery and validation, and monitoring drug treatment regimes.

The highly unstable nature of RNA transcripts *ex vivo* creates an even greater challenge for accurate analysis. Ongoing metabolic changes can drastically affect gene expression profiles in timeframes as short as one hour in "standard" blood-collection tubes post-draw (see Figure 1). Because blood is often drawn at sites remote from where the research is being conducted, there is often a time lag (typically hours but sometimes days) before the RNA isolation process can begin.

Various studies (references 1, 2 and Figure 1) show that among many factors, blood storage conditions can significantly alter gene expression results. Thus, for accurate measurement of gene expression profiles, blood must be drawn into a collection tube that contains an RNA stabilizing reagent.

The Tempus™ Blood RNA Tube system is designed for direct collection of 3 mL of blood into a plastic, evacuated blood collection tube containing the Applied Biosystems RNA Stabilizing Reagent. The RNA, and therefore the expression or transcript profile, is stable in the collection tube for up to five days at room temperature (18-25°C), and minimally 7 days at 4°C (Figure 3). The stabilized blood may also be frozen in the collection tube for prolonged storage or transport.

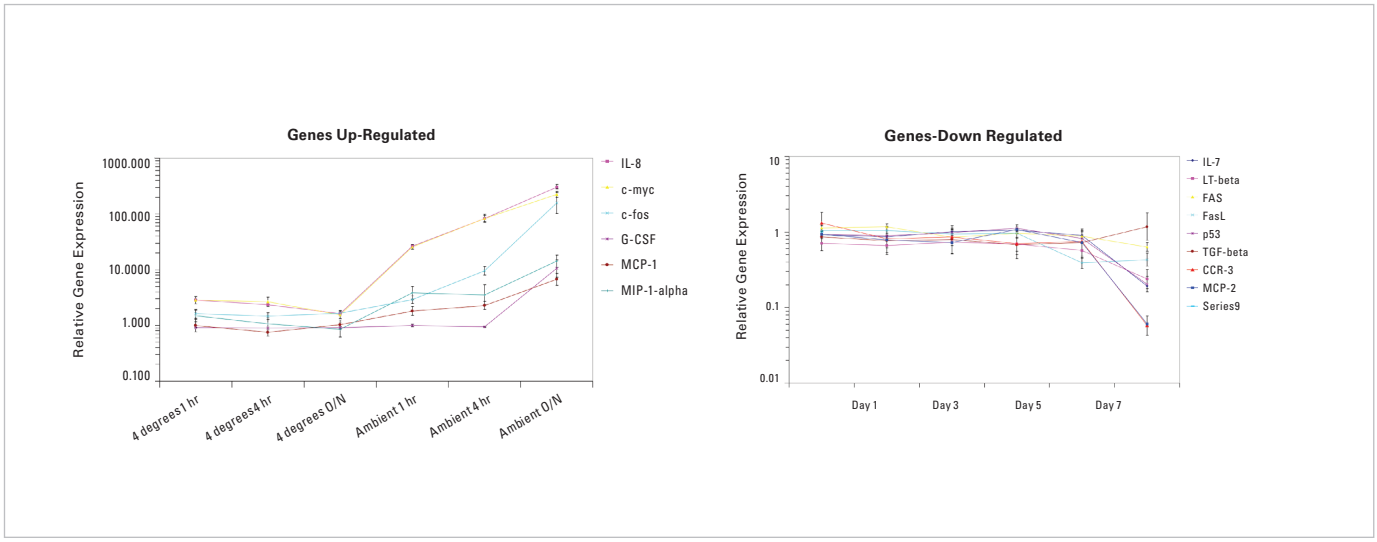


Figure 1. Gene expression stability in unstabilized whole blood (Tanner et al., *Clin. Lab. Haem.* April 2002, **24,** 337–341)

Tempus™ Blood RNA Tubes

The Tempus™ Blood RNA Tube was developed collaboratively by Applied Biosystems and Greiner Bio-One GmbH, one of the world's leading suppliers of blood collection products. Greiner Bio-One's VACUETTE® brand of blood collection tubes is well-known for quality and safety. These collection tubes were the first to be manufactured from the virtually unbreakable polyethylene terephthalate (PET) plastic.

The Tempus Blood RNA Tube is manufactured with a distinctive blue cap and black inner ring to distinguish it from other common venous blood collection tubes. It contains 6 mL of RNA Stabilizing Reagent and is calibrated to accurately draw 3 mL of whole blood.

When the blood is drawn into the tube and mixed with the reagent, lysis occurs immediately. The stabilizing reagent inactivates cellular RNases and selectively precipitates RNA, while genomic DNA (gDNA) and proteins remain in solution.

Tempus™ Spin RNA Isolation Kit

The Tempus Spin RNA Isolation Kit uses standard laboratory centrifuges and Ambion® glass-fiber filter cartridges to capture and purify high quality RNA.

The Tempus Spin RNA Isolation Kit workflow is easy to perform and can isolate RNA from up to 24 samples in approximately one hour (Figure 2). The Tempus workflow has fewer steps and does not require proteinase K or DNase treatment as compared to the leading competitor's lengthy, time-consuming process.

An optional on-filter DNase treatment (AbsoluteRNA Wash Solution) available separately, can be performed during the wash step to further reduce the trace amounts of DNA that might interfere with signal detection of low-expressing genes.

Performance with Ease

Even those new to the lab environment will find the Tempus Spin RNA Isolation Kit easy and simple to obtain quality results the first time (Table 1).

The combination of the Tempus Blood RNA Tubes and Tempus Spin RNA Isolation Kit provides greater RNA stability, high yields of purified RNA (observed yields of 6-25 µg from a full blood draw from normal, healthy donors), and high quality RNA (A260/280 ratio >1.9, RIN > 7.0) for downstream applications, such as quantitative PCR, microarray profiling, and other RNA analysis methods.

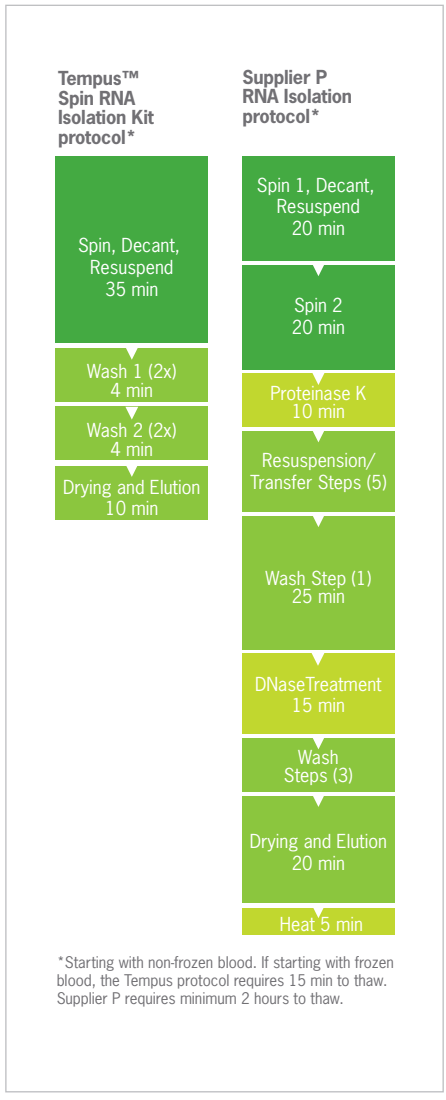


Figure 2. Comparison of Workflows for the Tempus™ Spin RNA Isolation Kit and Supplier P.

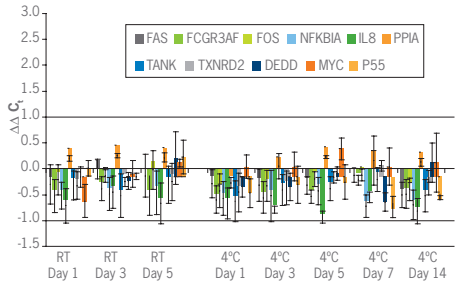
Transcript Stability

In addition to the ease of use in obtaining high yields of quality RNA, the Tempus™ system excels at stabilizing RNA expression profiles over a range of storage conditions.

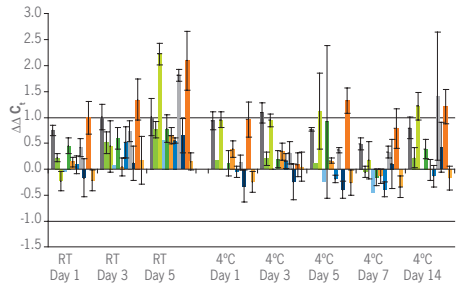
Replicate samples prepared immediately, or stored for 5 days at room temperature, were analyzed via microarrays using Tempus or Supplier P systems. Figure 4 shows high correlation in transcript profiles (r=0.9944) between Day 0 and Day 5 analyzed blood. A larger variation between days, as well as replicates, is observed when following Supplier P's recommended protocol. This data is supported by quantitative real-time PCR of samples stored at both room temperature and 4°C for both Tempus and Supplier P systems (Figure 3).

TABLE 1. TEMPUS™ SPIN RNA ISOLATION KIT OVERVIEW

Yield	6-25 µg/tube (2-8 µg/mL blood)
Quality	RIN > 7.0 A260/280 > 1.90
Concentration	> 60 ng/µL
Gene Expression Stability	5 days at room temperature ≥ 7 days at 4°C
Extraction Time	Approx. 1 hour/up to 24 samples
gDNA contamination	<0.05% by weight (-DNase treatment) <0.002% by weight (+DNase treatment)
Equipment Needed	Refrigerated centrifuge capable of 3,000xg with 50 mL-conical tube adapters Tabletop microcentrifuge capable of 16,000xg with microcentrifuge tubes

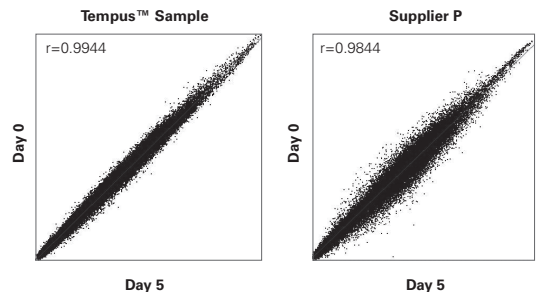


Panel A. Real-Time RT-PCR Using Tempus™ Sample



Panel B. Real-Time RT-PCR Using Supplier P Sample

Figure 3. Temporal Analysis of Gene Expression Profiles Obtained from Samples Using Tempus™ Blood RNA Tubes or Supplier P Tubes. Blood samples collected in Tempus Blood RNA Tubes or tubes from Supplier P were processed on the blood collection day (day 0), stored at room temperature from 1 to 5 days, or stored at 4°C for 1 to 14 days before RNA isolation using the recommended protocols from Applied Biosystems or Supplier P. Eleven TaqMan® Gene Expression Assays were used to examine RNA stability over time (compared to day 0) with the two different blood RNA processing systems. Tempus RNA samples (Panel A) were more stable over storage time compared to Supplier P RNA (Panel B). RT=room temperature



	Mean Technical Replicate Correlation	Mean Within Kit/ Between Day Correlation
Tempus™ Sample, Day 0	0.9957	0.9944
Tempus™ Sample, Day 5	0.9955	
Supplier P Sample, Day 0	0.9937	0.9844
Supplier P Sample, Day 5	0.9889	

Figure 4. Microarray Results Obtained Between RNA Isolation and Storage Time from Blood Samples Using Tempus™ Blood RNA Tubes or Supplier P Tubes. Blood samples were collected in Tempus Blood RNA Tubes or tubes from Supplier P. Sample tubes were processed on the collection day or stored at room temperature for 5 days before RNA isolation using the recommended protocols from Applied Biosystems or Supplier P. After processing all RNA samples with the GLOBINclear™-Human Whole Blood Globin Reduction Kit (Cat# AM1980), samples were amplified and labeled with the MessageAmp™ II-Biotin *Enhanced* Kit (Cat# AM1791). A summary of results from gene expression microarrays (Affymetrix® GeneChip) indicates that triplicate samples processed with the Tempus System show higher correlation between technical replicates and storage time points compared to triplicate samples processed with the Supplier P kit.

References

1. Tanner MA, Berk LS, Felten DL, Blidy AD, Bit SL, Ruff DW. Substantial changes in gene expression level due to the storage temperature and storage duration of human whole blood. *Clin Lab Haematol* 2002; **24:**337-341.

2. Rainen L, Oelmuller U, Jurgensen S, Wyrich R, Ballas C, Schram J, et. al. Stabilization of mRNA expression in whole blood samples. *Clin Chem* 2002; **48:**1883-1890.

ORDERING INFORMATION

Description	Size	P/N
Required Consumables and Reagents		
Tempus™ Blood RNA Tubes	50 tubes	4342792
NEW Tempus™ Spin RNA Isolation Kit	50 preps	4380204
NOTE: VACUETTE® Safety Blood Collection Sets for blood collection are not supplied by Applied Biosystems and must be ordered directly from Greiner Bio-One GmbH or your Greiner Bio-One distributor. Please refer to www.gbo.com/en for more information.		
Optional Consumables and Reagents		
AbsoluteRNA Wash Solution (DNase treatment)	10 mL	4305545
2-mL Collection Tubes (extra tubes)	100 count	AM12480
GLOBINclear™ Whole Blood Globin Reduction Kit – Human	20 rxn	AM1980
6 Tube Magnetic Stand (for use with GLOBINclear™)	1 unit	AM10055
50-mL Conical Tubes	200 count (racked) 250 count (bagged)	AM12501 AM12502
Alternative Consumables and Reagents		
Tempus™ 12-port RNA Isolation Kit (for use on 6100 Nucleic Acid PrepStation)	24 preps	4378672

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