BECKMAN COULTER
Life Sciences

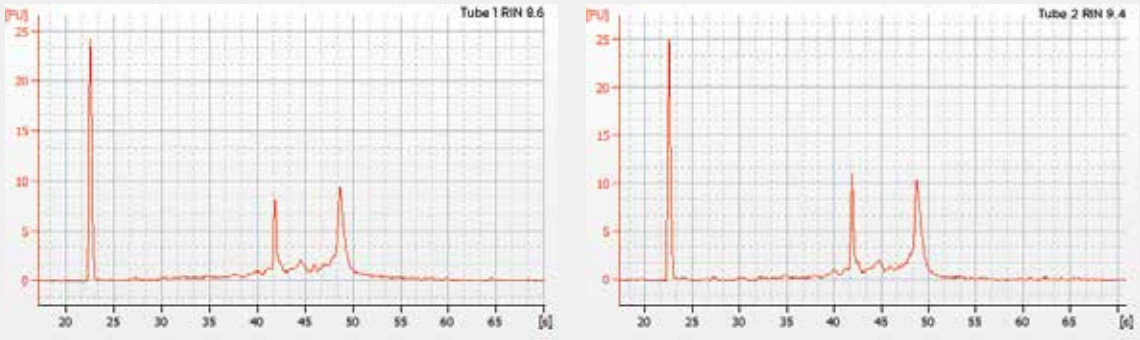
RNA Extraction from RNA-Stabilizing Blood Collection Tubes

RNAdvance Blood

The RNAdvance Blood kit is a ribonucleic acid (RNA) isolation reagent kit built on SPRI paramagnetic bead-based technology. It enables purification of high quality RNA with demonstrated compatibility up to 400 µL of blood collected in PAXgene RNA tubes. The extraction can be run manually in a 2 mL tube format or 96-well format, or automated in 96-well format on variety of Beckman Coulter Biomek liquid handling workstations. Total RNA extracted from PAXgene-preserved blood using the RNAdvance Blood kit is free of detectable gDNA.

- Compatible with downstream gene expression analysis techniques:
  - NGS
  - qRT-PCR
  - Microarray
- Produces high quality RNA
- Efficient removal of genomic DNA and other contaminants

RNA Integrity consistent among samples



Sample	RIN
Tube 1	8.6
Tube 1	9.5
Tube 1	9.4
Tube 2	9.4
Tube 2	9.9
Tube 2	9.3
Tube 3	9.8
Tube 3	9.9
Tube 3	8.7

Figure 1. Samples were taken from three different tubes taken from the same donor on the same day. All samples were prepped within 6 hours of blood draw and blood was kept on ice or at 4°C before RNA was extracted. The RIN scores averaged 9.4 with a σ2 of 0.4 for the 9 samples prepped. The RIN scores were evaluated by using a Bioanalyzer 6000 RNA Nano Assay (Agilent).

RNAdvance Blood isolates RNA at a higher yield than other suppliers

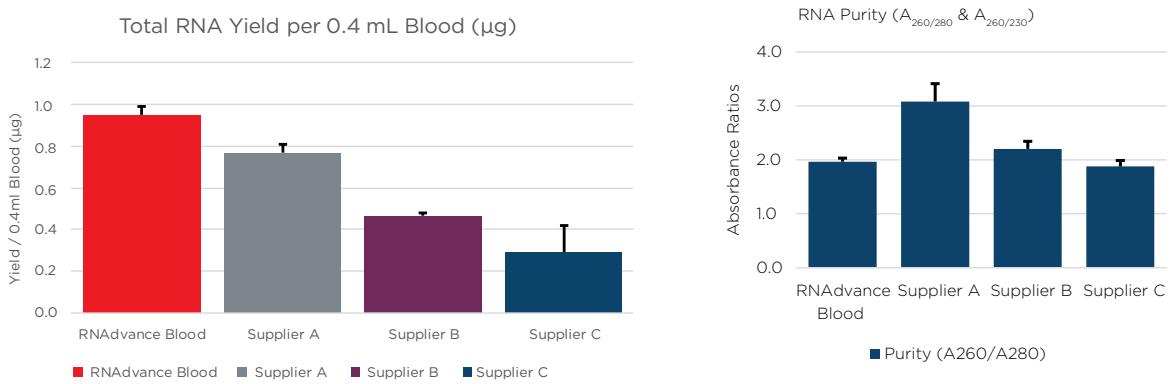


Figure 2. (Left) Samples were quantified using the NanoDrop (Thermo Fisher Scientific). RNAdvance Blood kit isolated two fold more RNA than kits from suppliers' B and C. (Right) Samples were assessed for purity using the NanoDrop (Thermo Fisher Scientific). Error bars represent the standard deviation of three technical replicates. RNAdvance Blood isolated RNA suitable for use in downstream applications.

RNAdvance provides consistent performance

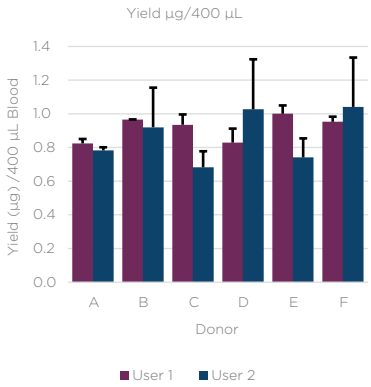


Figure 3. RNA was extracted from 6 donors from 2 different users within 24 hours. Samples were quantified using the NanoDrop (Thermo Fisher Scientific). Both users extracted similar total amounts of RNA. Error bars represent the standard deviation of three technical replicates.

RNAdvance eliminates DNA and PCR inhibitors for use in downstream applications

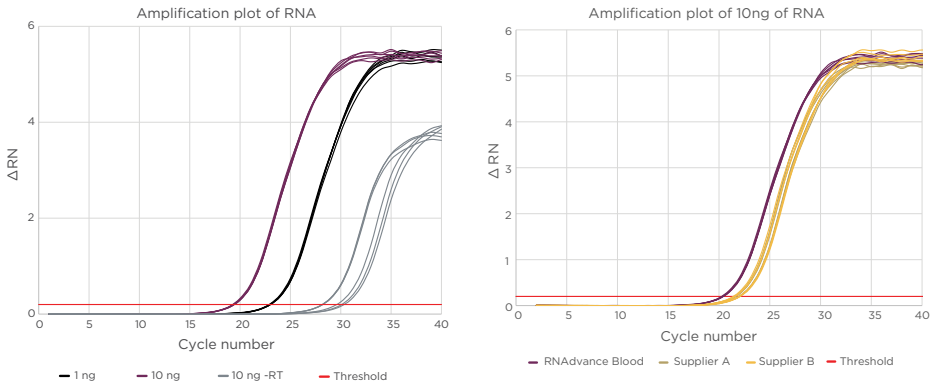


Figure 4. The ablility to PCR was assessed via qRT-PCR using a primer set (forward primer 5´-ggacttcgagcaagagatgg-3´ and reverse primer 5´-agcactgtgttggcgtacag-3´) designed to span Exon 4 and 5 of the beta (β)-actin gene (ActB) to produce 327 base pair amplicons. (Left) The no RT control also demonstrates the removal of DNA that can interfere with downstream RNA applications. (Right) The RNA isolated using the RNAdvance Blood kit was amplifiable indicating that the kit removed PCR inhibitors.

Visual Workflow

1. Lyse blood collected in PAXgene tubes in Lysis Buffer and proteinase K
2. Bind RNA to magnetic beads
3. Separate magnetic beads from contaminants
4. Wash magnetic beads with Wash Buffer and 70% ethanol to remove contaminants
5. Treat samples with DNase I
6. Rebind RNA to magnetic beads with Bind 2 Buffer
7. Wash magnetic beads with 70% ethanol to remove contaminants
8. Elute RNA from magnetic beads
9. Transfer to new plate

RNAdvance isolates high amounts of RNA at high quality from blood collected in PAXgene tubes for up to 4 days

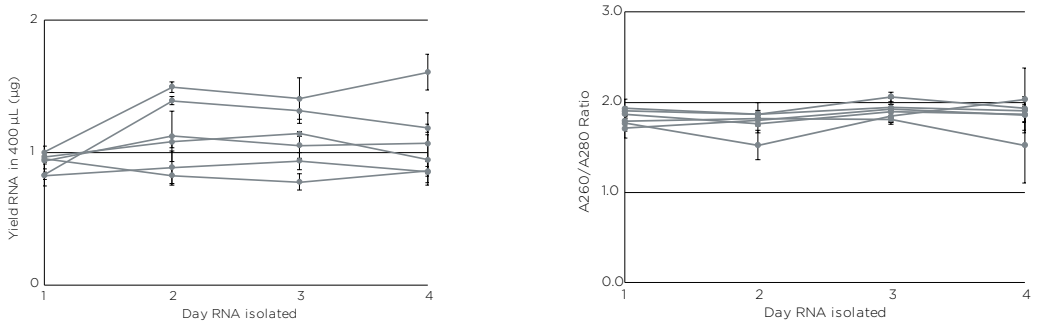


Figure 5. RNA was extracted from 6 donors for 4 days after donation. The blood was collected in PaxGene RNA tubes and were stored at 4°C. (Left) RNA yields were consistently 1 µg of RNA from 400 µL of blood. There was more donor variance at the Day 2- Day 4 extraction than at Day 1. (Right) Purity did not change significantly throughout the 4 days and were acceptable for downstream applications. Error bars represent the standard deviation of three technical replicates.

Extract RNA from samples in less time with less pipette actions compared to users of column based kits

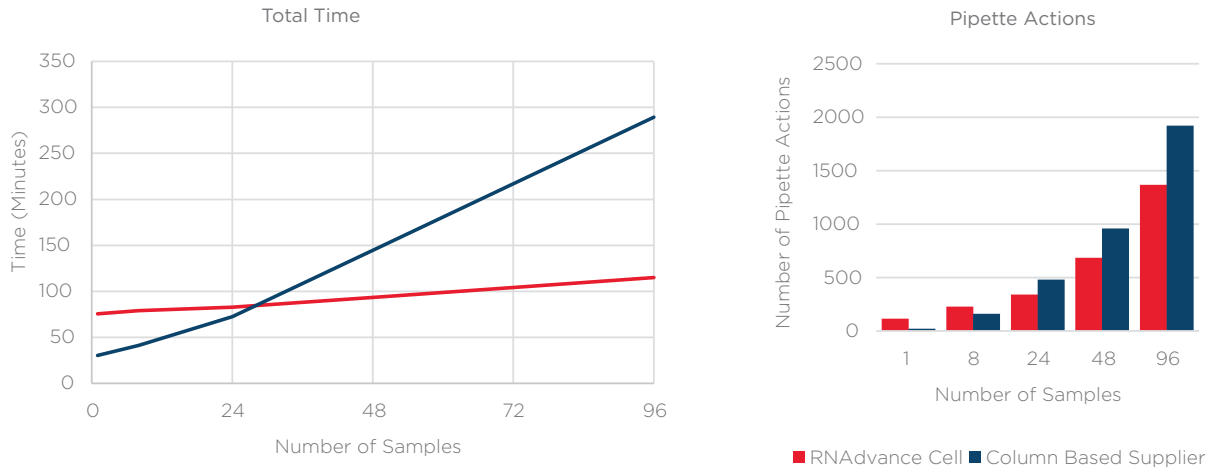


Figure 6. (Left) Represents total time to extract RNA for 1 to 96 samples using RNAdvance Blood or a column based supplier. Even at 15 samples total time to extract RNA from blood is faster using RNAdvance Blood. (Right) The total number of pipette actions, which include dispensing a sample, mixing a sample and discarding tips, required for 1, 8, 24, 48, and 96 samples. With the ability to use a multichannel pipette there is significantly less pipette actions that need to take place than with column based suppliers.

For use in manual or automated methods based on batch size or overall throughput

			RNAdvance Blood	
			Manual	Automated
Batch Size	8	Hands-on Time	1.00	0.25
		Total Time	2.50	2.5
	24	Hands-on Time	1.50	0.25
		Total Time	3.00	2.5
	48	Hands-on Time	NR	0.25
		Total Time	NR	2.5
	96	Hands-on Time	NR	0.25
		Total Time	NR	3.0

Table 1. Estimated hands-on time and total time in hours, required to perform 8, 24, 48 and 96 RNAdvance Blood RNA extractions The methods can be performed either manually or automated on a liquid handling system. Data represented in this table is based on a Biomek i7 Hybrid Genomics Workstation. Difference in time between manual and automation is indicated. NR=Not Recommended.

Product infoRMATION

PART NO	NAME	PREPS
A35603	RNAdvance Blood Kit	50
A35605	RNAdvance Blood Kit	96
A35604	RNAdvance Blood Kit	384

For more information, please contact:

For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at beckman.com

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