

RNA Complete BCT™ technical product note

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Intended use

RNA Complete BCT (RNAC) maintains draw-time concentrations of extracellular vesicles (EVs) and associated cell-free RNA (cfRNA).

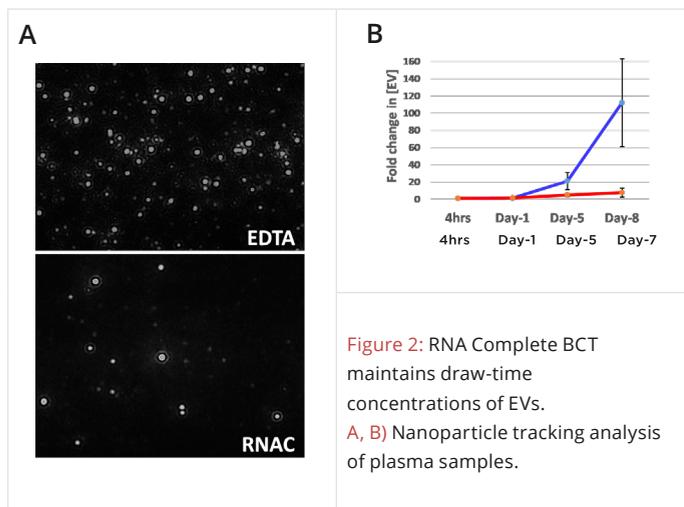
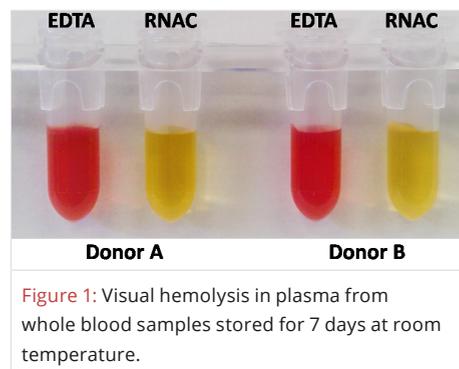
Background

Extracellular vesicles, such as exosomes and microvesicles, are shed by all cells. Current stabilization tubes, such as Streck's Cell-Free DNA BCT®, effectively stabilize white blood cells and hence draw-time levels of cell-free DNA. They do not, however, maintain the integrity of all cells in the collected blood sample. In order to maintain draw-time EV concentrations, Streck designed a novel blood collection tube targeting erythrocytes and platelets in addition to white blood cells. In parallel with maintaining draw time levels of EVs, the vesicular contents of the EV population are also maintained. These contents, importantly, contain circulating RNA like mRNA.

Supporting data

Plasma collection and processing

As noted in the IFU, a general double spin protocol is utilized for plasma preparation from the RNA Complete BCT (1800g initial and 2800g clarification). Other stabilization tubes on the market can show dramatically increased hemolysis and decreased plasma yields. RNA Complete BCT is designed to limit those changes (Figure 1).



Extracellular vesicle analysis

In order to assay the EV population, Streck utilizes the Malvern NanoSight NS300 instrument. Analysis using this system provides a nonbiased size-based assessment of EV concentration and particle size. While unstabilized blood samples demonstrate considerable donor-dependent increases in EV concentration as a function of room temperature storage time, the RNA Complete BCT maintains draw-time EV concentration out to 7 days when stored at room temperature (Figure 2).

Cell-free RNA isolation and analysis

The RNA Complete BCT is compatible with standard total plasma nucleic acid isolation kits such as those from Qiagen (QIAamp Circulating Nucleic Acid Kit), Norgen (Plasma/Serum Circulating and Exosomal RNA Purification Kit), and Applied Biosystems (MagMAX Cell-Free Total Nucleic Acid Isolation Kit).

The cfRNA isolations incorporate a DNase1 digestion step to remove any contaminating DNA co-isolated during the purification step. The cfRNA analysis is subsequently completed either by direct means, such as Agilent Bioanalyzer 2100 or TapeStation, or indirectly using reverse transcription followed by PCR using the Bio-Rad QX200 Droplet Digital PCR System (Figure 3). Robust increases in cfRNA levels are observed as a function of storage time when drawn into non-stabilizing tubes, however, remain at draw-time concentrations when drawn into the RNA Complete BCT.

Next-generation sequencing

One reason for development of the RNA Complete BCT was next-generation sequencing workflows. Specifically, maintaining draw-time cfRNA transcript levels was sought for whole blood samples for use in transcriptome studies. Cell-free RNA isolated from the RNA Complete BCT is compatible with standard low input library prep kits, such as those manufactured by New England BioLabs (NEBNext Ultra II Directional RNA Library Prep Kit) and Illumina (TruSeq RNA Library Prep Kit).

Testing utilizes Illumina sequencing methods with ribosomal RNA-depleted samples. While unstabilized blood samples demonstrate dramatic changes in transcript abundance as a function of storage time, samples drawn into the RNA Complete BCT maintain transcriptome profiles similar to the initial blood draw (Figure 4).

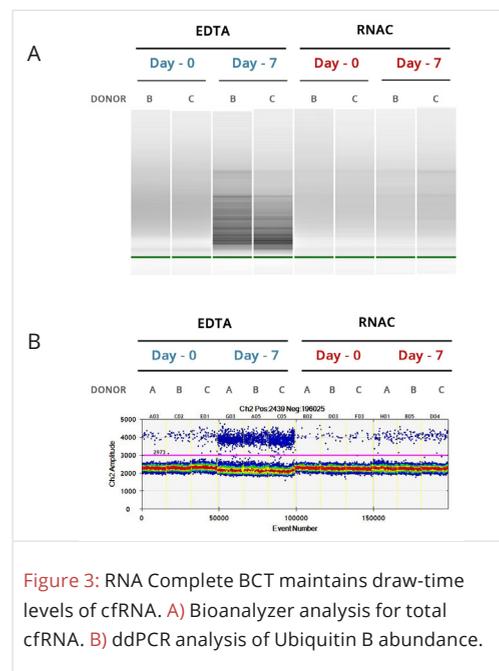


Figure 3: RNA Complete BCT maintains draw-time levels of cfRNA. A) Bioanalyzer analysis for total cfRNA. B) ddPCR analysis of Ubiquitin B abundance.

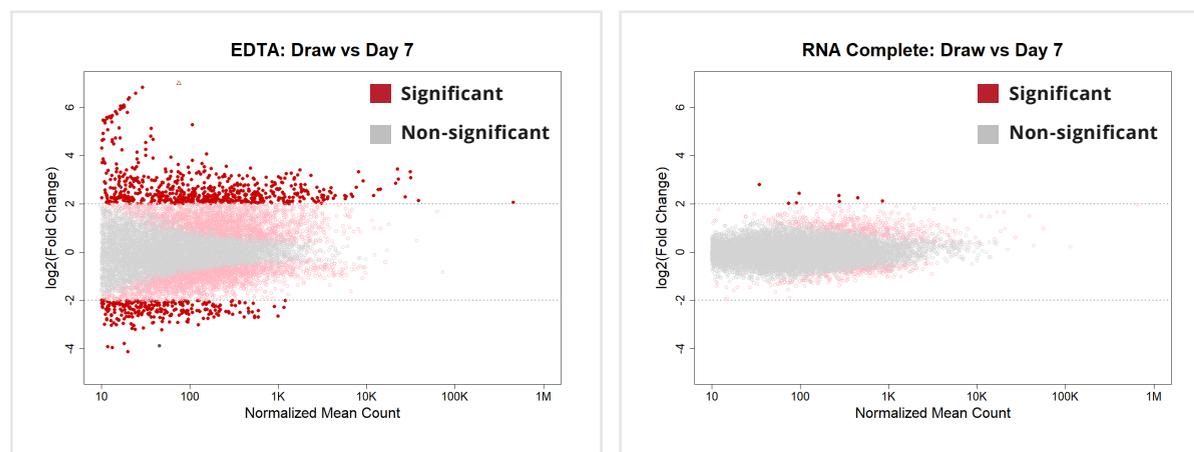


Figure 4: RNA Complete BCT maintains draw-time expression profile of collected blood samples for up to 7 days when stored at room temperature. DESeq2 analysis of samples obtained from a minimum of five independent healthy donors.

	EDTA Tubes	RNA Complete
Genes detected	11,970	12,063
Differentially expressed genes	928	8