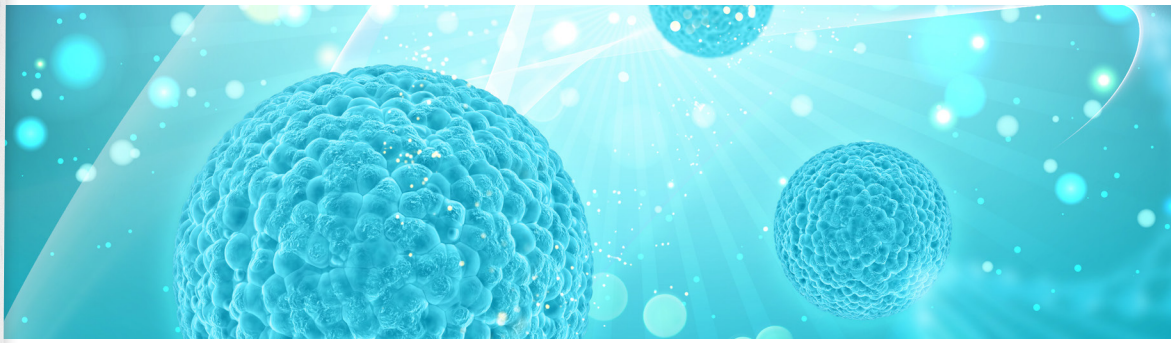
cf-DNA/cf-RNA Preservative Tubes

CAT. Dx63950,63950

PRESERVATION AND ISOLATION OF BOTH cf-DNA AND cf-RNA FROM A SINGLE TUBE



- ☑ Fixative-free preservative, no cross-linking of DNA
- ☑ Preserve cf-DNA/ct-DNA and cf-RNA for 30 days at ambient temperature and for up to 8 days at 37°C
- ☑ Preserve Circulating Tumour Cells (CTCs) for 14 days at ambient temperature
- ☑ No plasma volume loss after shipping/transportation
- ☑ Prevent hemolysis allowing better separation of plasma
- ☑ Prevent apoptosis of blood cells and fragmentation of genomic DNA
- ☑ Produce high quality/quantity of plasma cf-DNA/ct-DNA/cf-RNA
- ☑ The procedure for plasma isolation using this kit is compliant with ISO 20186-3:2019
- ☑ Available in CE-IVDR Format

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Evaluation of commercial tubes for the preservation of cf-DNA/ ct-DNA & cf-RNA

HIGH cf-DNA QUANTITY FROM NORGEN'S PRESERVED BLOOD

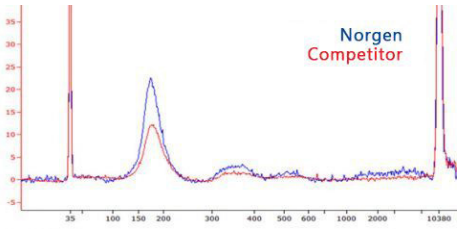
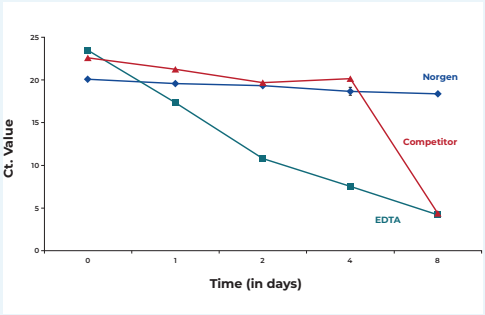


Figure 1. High Quantity of cf-DNA from Plasma preserved using Norgen's cf-DNA/cf-RNA Preservative Tubes. Blood samples from the same donor were drawn into either Competitor tubes or Norgen's cf-DNA/cf-RNA Preservative Tubes and stored at room temperature for 7 days. Norgen's cf-DNA/cf-RNA preservative Tube recovered 6.5mL plasma whereas the Competitor's tube recovered 3.5mL plasma. The cf-DNA was then isolated from the entire plasma volume recovered from each tube using Norgen's Plasma/Serum Cell-Free Circulating DNA Purification Midi Kit (Cat. 55600) and Norgen's Plasma/Serum Cell-Free Circulating DNA Purification Maxi Kit (Cat. 55800). The quantity of DNA recovered from both samples were assessed using the Agilent Bio-analyzer High Sensitivity DNA Chip. As can be seen in the bioanalyzer trace,Norgen's preservative tube yielded more cf-DNA (Blue peak) as compared to the cf-DNA recovered from the Competitor's preservative tube.

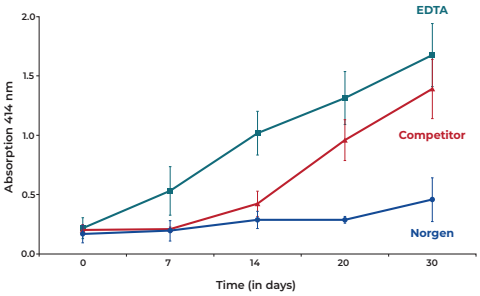


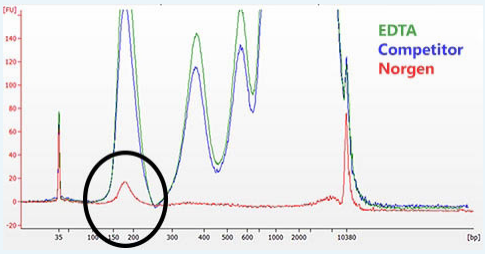
STABLE AT HIGH SHIPPING TEMPERATURES (37°C)

Figure 2. Effect of high temperature (37°C) storage for 8 days. Blood samples were drawn into either EDTA tubes, Competitor tubes or Norgen's cf-DNA/cf-RNA Preservative Tubes and stored at 37°C. cf-DNA was then isolated from processed plasma. gDNA concentration was determined by real-time PCR using a long ALU gene target (247 bp). The gDNA - target in EDTA tubes showed a significant drop in the Ct.value after 1 day of storage at 37°C compared to the initial time point, and continued to drop until the 8th day of storage. For Competitor, the gDNA - target remained stable up to the 4th day of storage and then the Ct. values started to significantly drop, indicating poor stabilization beyond day 4. Norgen's cf-DNA/cf-RNA Preservative Tubes stabilized samples for 8 days.

PREVENT HEMOLYSIS

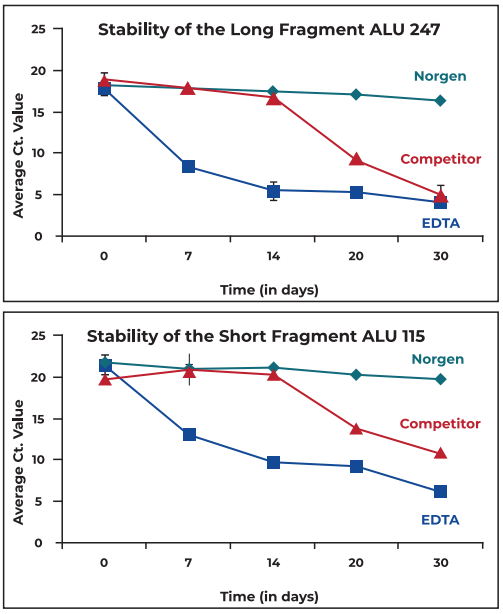
Figure 3. Hemolysis of collected blood measured over time. Blood samples were drawn into: 1) EDTA tubes, 2) Competitor tubes and 3) Norgen's cf-DNA/cf-RNA Preservative Tubes and stored for up to 30 days. Hemolysis was determined by measuring the absorption of free hemoglobin in plasma from 3 subjects at 414 nm over several time points. Mean absorption is shown. The amount of free hemoglobin increased rapidly with each additional storage day in the EDTA tubes and Competitor tubes, and remained relatively constant in Norgen's cf-DNA/cf-RNA Preservative Tubes.

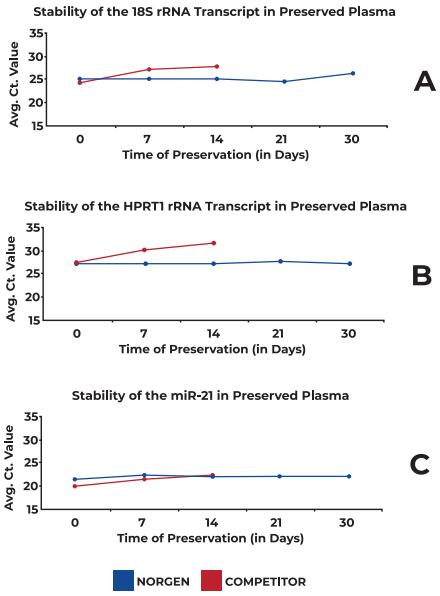




PREVENT gDNA RELEASE INTO PLASMA DURING SHIPPING/TRANSPORTATION

Figure 4. Prevent cell lysis and the release of gDNA and accumulation of apoptotic ladder in plasma. Blood samples drawn into three different tubes (Norgen's cf-DNA/cf-RNA Preservative Tubes, EDTA, and Competitor) and stored for up to 30 days. Norgen's cf-DNA/cf-RNA Preservative Tubes helps prevent the release of high molecular weight gDNA into plasma while also minimizing the accumulation of contaminating apoptotic ladder from dying peripheral blood leukocytes. As indicated in the black circle, gDNA contamination is at a very low level as compared to both the Competitor and EDTA tubes. This is indicative of very low levels of cell lysis and subsequent release of gDNA into the preserved plasma sample.





cf-DNA STABLE FOR **30 DAYS** AT AMBIENT TEMPERATURE

Figure 5. Effect of ambient temperature storage on cf-DNA (pDNA), exemplified by the short Alu (115bp) fragment, and genomic DNA (gDNA), exemplified by the large Alu (247 bp) fragment. Blood samples were drawn into either: 1) EDTA tubes, 2) Competitor tubes or 3) Norgen's cf-DNA/cf-RNA Preservative Tubes and stored at room temperature. Aliquots of blood were removed at the indicated times and the plasma was separated. DNA was isolated, and pDNA and gDNA concentrations were determined by real-time PCR using a short ALU gene target (115 bp) representing the pDNA and a long ALU gene (247 bp) representing the gDNA. Levels of these two fragments should stay the same for the duration indicating stabilization and no hemolysis. As expected, there was no stabilization of cf-DNA and extensive hemolysis in the EDTA tube. Competitor showed significantly lower Ct values for both genes after 14 days, whereas cf-DNA was stable for 30 days at room temperature for Norgen's cf-DNA/cf-RNA Preservative Tubes.

cf-RNA STABLE FOR **30 DAYS** AT AMBIENT TEMPERATURE

Figure 6. Effect of ambient temperature storage on cf-RNA, exemplified by the 18S rRNA transcript, HPRT1 mRNA transcript and miR-21. Blood samples were drawn into either: 1) Competitor tubes or 2) Norgen's cf-DNA/cf-RNA Preservative Tubes. Competitors tubes were stored at room temperature for 14 days whereas Norgens cf-DNA/cf-RNA Preservative Tubes were stored for 30 days at room temperature. At the indicated times each tube was processed and the plasma was separated. Cf-RNA was isolated and the stability of the purified cf-RNA was determined by rt-qPCR amplification targeting the 18S rRNA transcript, HPRT1 mRNA transcript and miR-21. Levels of these three targets should stay the same for the duration indicating stabilization. Competitor showed significantly higher Ct values for the three targets after 7 days indicating cf-RNA degradation, whereas cf-RNA was stable for 30 days at room temperature for Norgen's cf-DNA/cf-RNA Preservative Tubes.

Maximum Plasma Volume Recovery After Shipping

BEFORE SHIPPING

AFTER SHIPPING

No plasma volume loss after shipping/transportation. Blood was drawn from 6 different donors in duplicate. One set was kept in the lab at room temperature and the other was packed in an insulated box and shipped from Thorold, ON via overnight air freight to Winnipeg, MB and then back to Thorold ON (elapsed time 72 h). Upon return, preserved samples were stored at room temperature for 7 days before plasma was separated. The plasma volume recovered from Norgen's cf-DNA/cf-RNA Preservative Tubes did not change before shipping or after shipping (6-7 mL recovered plasma). For both Competitor tubes and EDTA tubes the plasma volume recovered before shipping was ~ 4 mL and after shipping was ~ 2.5 mL.

Select Publications

Publication Title	Authors	Journal	Year
Circulating tumor DNA profiling for childhood brain tumors: Technical challenges and evidence for utility	Liu, A. P.-Y., Northcott, P. A., Robinson, G. W., & Gajjar, A.	Laboratory Investigation, 102(2), 134–142.	2022
From Sampling to Sequencing: A Liquid Biopsy Pre-Analytic Workflow to Maximize Multi-Layer Genomic Information from a Single Tube	Maass, K. K., Schad, P. S., Finster, A. M. E., Puranachot, P., Rosing, F., Wedig, T., Schwarz, N., Stumpf, N., Pfister, S. M., & Pajtler, K. W.	Cancers, 13(12), Article 12	2021
Evaluate and Optimize Cell-Free RNA Extraction Methods to Apply for Alzheimer's Disease Biomarkers Detection	Le, A. P. H., Tran, T. T., Cao, T. H. M., Le, T. M., Le, P. T., & Huong, H. T. T.	8th International Conference on the Development of Biomedical Engineering in Vietnam (pp. 591–609). Springer International Publishing.	2021
Evaluation of Storage Tubes for Combined Analysis of Circulating Nucleic Acids in Liquid Biopsies	Ward Gahlawat, A., Lenhardt, J., Witte, T., Keitel, D., Kaufhold, A., Maass, K. K., Pajtler, K. W., Sohn, C., & Schott, S.	International Journal of Molecular Sciences, 20(3), Article 3.	2019

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Norgen's preservation technology is patent pending.

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