APPLICATION NOTE

cfDNA extraction from freeze-dried plasma samples

Using 300K Solutions Plasma and Serum Stabilization Kit

► Introduction

The term liquid biopsy (LB) refers to molecules such as proteins, DNA, RNA, cells, or extracellular vesicles that are present in blood and other bodily fluids that originate from the primary and/or metastatic tumor. LB could provide in certain situations an alternative to solid biopsy since would allow real-time monitoring of a tumor via a minimally invasive sample extraction, such as blood.

To achieve a reliable analysis of these samples to be used in downstream applications, the preanalytical procedures (collection, quality and storage) should be carefully considered and strictly followed.

The standardized storing protocol of plasma and serum samples used for downstream liquid biopsy analysis based on circulating-free DNA (cfDNA) is the ULT storage (-80 ºC). Here, *300K Solutions* presents an alternative for stabilization and storage at room temperature (RT) for these plasma and serum samples.

► Materials and methods

This study was performed in collaboration with the Molecular Biology Laboratory of Hematology unit at the Salamanca University Hospital.

Plasma samples were obtained from peripheral blood following strictly the preanalytical procedures for the handling of these type of samples. Then, 1.5 mL plasma aliquots from each patient were stored at -80 ºC.

After their storage, one aliquot of each patient was thawed and lyophilized using the Plasma and Serum Stabilization Kit 24 of *300K Solutions*. *[Fig. 1].*

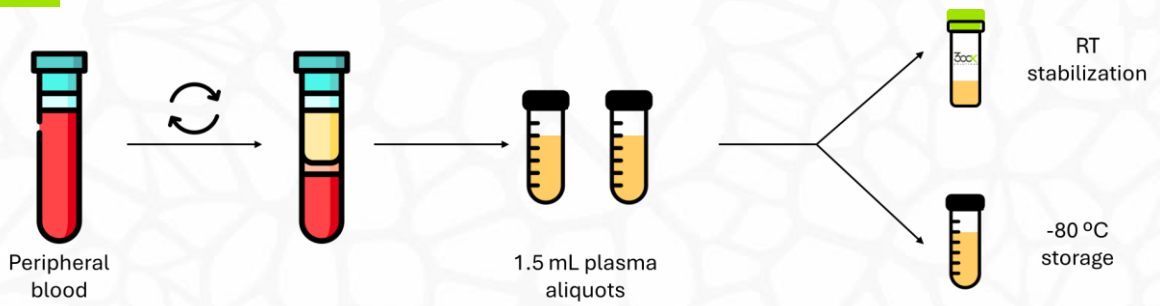

***Fig 1**. Study design flowchart*

For freeze-drying stabilization using *300K Solutions* technology, the samples were added to a vial containing the specific plasma buffer provided with the kit. Freeze-drying was performed using the Sample Stabilization System (S3) which oversees the entire lyophilization process to ensure the optimal efficiency *[Fig. 2].*

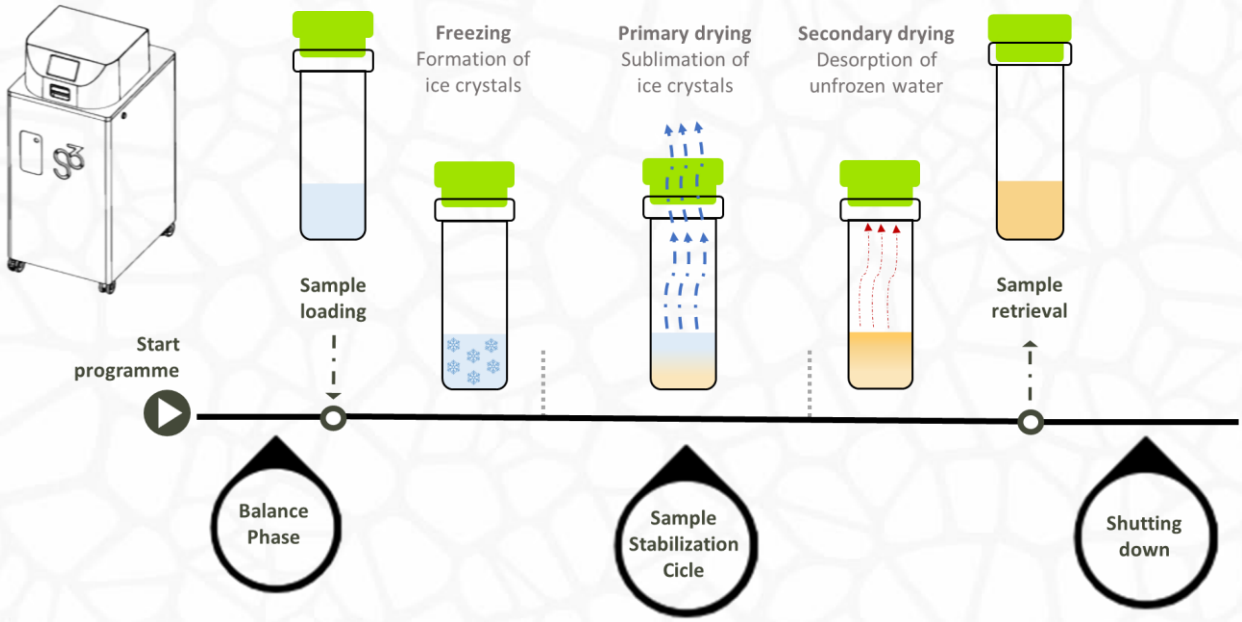

***Fig 2**. Freeze-drying process carried out by the Sample Stabilization System (S3).*

Following the freeze-drying process, samples were rehydrated and subjected to cfDNA extraction using the QIAamp MinElute ccfDNA Mini Kit (Qiagen). At the same time, cfDNA of frozen samples were obtained to compare both storage methods.

Finally, extracted cfDNA was then subjected to a basic quality control (QC):

- DNA quantity by fluorimetry (Qubit).
- DNA integrity analysis by Agilent 2200 TapeStation System from Agilent Technologies.

► Results

QC assessment performed in cfDNA samples stored at room temperature (RT) and at -80 ºC showed similar results in terms of quantity and quality. *[Fig. 3]. [Table 1].*

Sample	[cfDNA] -80ºC storage	[cfDNA] RT storage
1	0.282 ng/µL	0.314 ng/µL
2	0.126 ng/µL	0.170 ng/µL
3	0.108 ng/µL	0.228 ng/µL
4	0.270 ng/µL	0.142 ng/µL

***Table 1**. Concentration of the different cfDNA samples measured by fluorimetry (Qubit)*

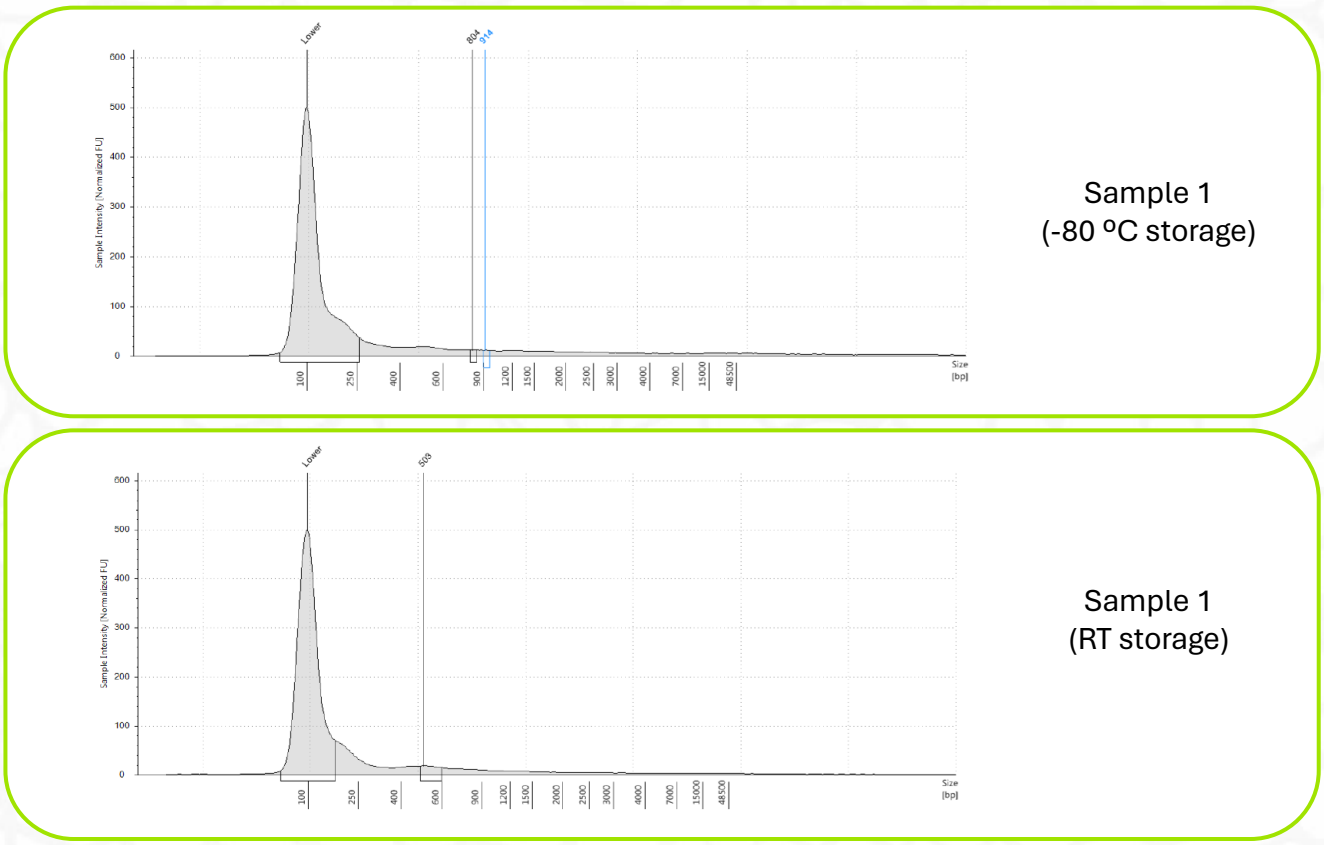

***Fig 3**. Visual TapeStation results of cfDNA extracted from samples stored at -80ºC or at RT corresponding to sample 1*

► Conclusions

1. cfDNA is preserved in plasma samples that have been stabilized with the Plasma and Serum Stabilization Kit 24.
2. The quantity and quality of cfDNA extracted from both lyophilized samples (using the Plasma and Serum Stabilization Kit 24) stored at RT and samples stored at -80 ºC is comparable.

►Reference

A Standardized Liquid Biopsy Preanalytical Protocol for Downstream Circulating-Free DNA Applications (doi: 10.3791/64123)

Liquid biopsy: a non-invasive approach for Hodgkin lymphoma genotyping (doi: 10.1111/bjh.17719)

Images: *Flaticon*