

Biotype Diagnostic GmbH

Technical Report



Samplettype **i-sep**[®] DL and SQ -
Outstanding performance for sample lysis,
incubation and separation of DNA-lysates in
the laboratory

Bio **type**[®]

Biotype Diagnostic GmbH



Samplettype **i-sep**[®] DL

Differential Lysis - gradual DNA extraction the easy way

Abstract

The quantity and quality of genomic DNA extracted from any sample can greatly impact the success of the downstream analysis and the overall quality of the final result. This is particular the case for field specimens which might be limited in quantity, may be environmentally exposed, and may require purification from difficult substrates containing PCR inhibitors. Samplettype **i-sep**[®] DL are developed for multistage DNA extraction procedures to provide purified DNA lysates of highest quality. They are based on the established mini spin-column format containing a unique sealed filter compartment which prevents flow-through of different kinds of liquid without any centrifugal force. Sample lysis is carried out in the same column and lysis buffers are quantitatively transferred with no extra handling steps. Solutions are retained within the column even during heat incubation and moderate shaking. Upon centrifugation, the solution passes the column into the collection tube. Spin column systems that are used for this purpose should be safely and tightly sealed. Especially in terms of the protection against evaporation during sample incubation and the stability of the membrane, the experiments shown here confirm that Samplettype **i-sep**[®] spin column systems are fully suitable for these requirements. Thus, they are perfectly designed for safety and performance in demanding applications.

Introduction

Samplettype **i-sep**[®] extraction systems are made of highly pure polypropylene, a material that is mechanically stable and can be used in a broad temperature range while also being very resistant to chemicals. The single tube assembly and lid geometry is designed to minimize sample loss through evaporation and to prevent the opening of the lid at high temperatures.

This technical report describes four experiments designed to evaluate the properties of Samplettype **i-sep**[®] systems and their suitability to carry out the sample incubation (lysis) and DNA-separation (lysate) in a single tube assembly. The closure of the lid at 100°C, the membrane stability (sealing of the reaction chamber), and the seal tightness of the spin column systems in an evaporation test as well as the DNA recovery rate was examined.

Methods and Results

20 Samplettype **i-sep**[®] DL and Samplettype **i-sep**[®] SQ each were used in the experiments.

Seal safety

Filter columns were placed in a collection tube and filled with 0.5 ml aqueous solution. They were incubated for 30 min at 100°C in a water bath. Then, the number of opened lids was determined for each tube type.

During incubation in a boiling water bath, the Samplettype **i-sep**[®] systems remained safely closed. In 55% (11 vials) the lid was minimally lifted but safely sealed.

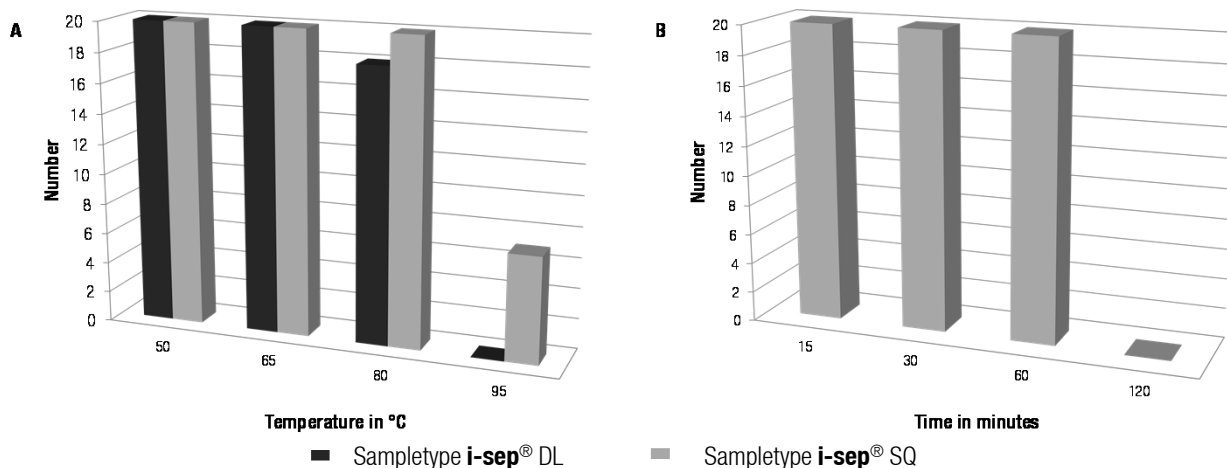


Figure 1: Stability testing of the membranes - (A) Tightness of the reaction chamber subject to temperature during an incubation time of 2 hours. (B) Tightness of the reaction chamber at 95°C subject to incubation time (Samplettype **i-sep**[®] SQ only).

Samplettype **i-sep**[®] SQ

Effective DNA preparation even from low sample inputs

Vapor tightness

To determine the vapor tightness of the systems the evaporation rate at higher temperature was tested. After placing filter columns in a collection tube and filling them with 0.5 ml aqueous solution, they were incubated for 1 h at 56°C. In a second independent experiment the samples were incubated overnight (16 hours) at 56°C (Block heater, Eppendorf, Thermomixer comfort). Weighing out before and after incubation (Laboratory scale, Sartorius, LA230S) determined the quantity of evaporated liquid.

The volume of evaporated liquid was 2 - 6 µl after one hour at 56°C. This corresponds in mean with less than 1% reduction of the aqueous solution. The loss of aqueous solution in the experiment at 56°C overnight was determined between 50 and 100 µl; corresponding to an average of reduction of 14%.

Membrane stability

After placing filter columns in a collection tube and filling them with 0.5 ml aqueous solution, they were incubated in a block heater (Eppendorf, Thermomixer comfort) for 2 h at 50°C, 65°C, 80°C and 95°C. In a second experiment Samplettype **i-sep**[®] SQ were incubated at 95°C for 15, 30, 60 and 120 minutes. In both experiments the stability of the membrane was determined by checking the tightness of the reaction chamber.

Figure 1 shows the temperature stability of the reaction chamber (membrane). No liquid was detected in the collection tube. The membrane of the Samplettype **i-sep**[®] DL was shown to be stable up to 65°C. A membrane stability of even 95°C was determined for Samplettype **i-sep**[®] SQ (Fig. 1A). However, to guarantee a sealed reaction chamber the incubation time at 95°C should not extend 60 minutes (Fig. 1B).

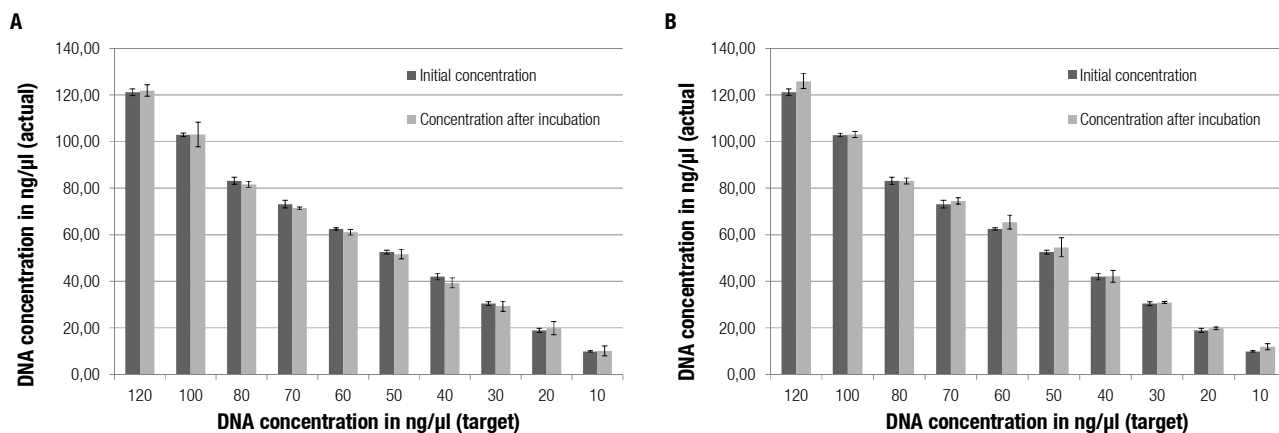


Figure 2: DNA-recovery rate of Samplettype **i-sep**[®] - (A) Recovery rate of Samplettype **i-sep**[®] DL (B) Recovery rate of Samplettype **i-sep**[®] SQ. Shown are mean values (n=2/6). Error bars indicate the standard deviation.

DNA-Recovery rate

To determine the retention capacity of membrane materials in terms of nucleic acid, ten different DNA concentrations were tested. A dilution series between 100 ng/µl and 10 ng/µl was prepared using the DNA reference standard (H7, *Candida krusei*; concentration c=2875.65 ng/µl). A photometric concentration determination was carried out in triplicates (Nanodrop ND-1000 Thermo Scientific; 1.5-2 µl sample). Two filter columns of Samplettype **i-sep**[®] DL and SQ, respectively, were filled with 0.5 ml of each dilution and incubated at room temperature for 30 minutes. The samples were centrifuged and the DNA concentrations were determined in triplicates again.

Mean values were calculated based on two membranes and the corresponding concentration measurements in triplicate. As shown in Figure 2 no DNA was retained by the membranes. DNA is transferred quantitatively into the collection tube for further processing.

Conclusion

Sampletype **i-sep**[®] systems are developed for quantitative DNA extraction procedures. The proprietary filter material allows the sample lysis and separation of DNA in the same device without any manual transfer of the substrate or additional pipetting steps. The novel column system is ideally suitable for DNA extraction procedures involving a diversion of specimens such as clinical or forensic sample material.

The results demonstrate that Sampletype **i-sep**[®] systems can be used for a broad spectrum of sample lysis methods.

The secure lid sealing and the small evaporation rates in the vapor tightness test confirm that Sampletype **i-sep**[®] systems are optimally suited for incubations at higher temperatures and over longer periods too. Using Sampletype **i-sep**[®] systems for preparing DNA extracts, the DNA released during sample lysis is quantitatively transferred into the collection tube during centrifugation. The DNA extracts produced can be used in the downstream workflow without loss. The combination of sample incubation (lysis) and the subsequent separation of the DNA lysate results in a product which is designed to be used in demanding laboratory applications and which provides maximum safety when working with valuable samples.

Order information

Product	Order number
Sampletype i-sep [®] DL (250)	60-00101-0250
Sampletype i-sep [®] SQ (500)	61-00201-0500
Sampletype i-sep [®] collection tubes (250)	62-00301-0250

For more information regarding our products or if you have any questions please do not hesitate to contact us.

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