

Applications

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Technical Report

Using Concentrator plus* in a sequencing laboratory

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Abstract

This Technical Report describes procedures that are routinely performed in a sequencing laboratory with the Concentrator plus, namely reconcentrating DNA samples, removing of alcohol residues, aliquoting enzymes, and using the Desiccator function.

It is shown that working with the Concentrator plus is easy and provides a fast and gentle evaporation.

By providing optimal evaporation conditions regarding the vapor pressure of the solvent and the temperature sensitivity of the samples, it saves time and prevents sample loss.

Introduction

Concentrator plus from Eppendorf was developed for the re-concentration of nucleic acids and proteins. It is thus ideal for high-throughput commercial sequencing labs since it is often necessary to process DNA that has too low a concentration for standard sequencing. Re-concentrating the sample using alternative methods – such as ethanol precipitation – is much more time-consuming and may lead to sample loss and thus to a quantity of DNA that is too low to enable error-free sequencing. The secret of drying DNA samples lies in striking exactly the right balance: if the sample contains too little moisture, it will be difficult to re-suspend the DNA, which will lead to a great deal of time being lost.

This application note includes a field report on the performance of Concentrator plus in a sequencing lab. This report clearly demonstrates that the Concentrator is suitable not only for re-concentrating DNA samples, but also for removing alcohol residue, aliquoting enzymes and as a desiccator for large tubes.



eppendorf

*Product Name in North America: Vacufuge® plus

Methods and Results

As a rule, sequencing reactions are prepared in 0.2 ml tubes. To process them as efficiently as possible in the rotors of the Concentrator, the adapters used are the same as those for Eppendorf centrifuges (Eppendorf adapter for 0.2 ml PCR tubes, see order information).

1. Re-concentrating DNA samples

Insufficient concentrations of DNA samples frequently lead to unsuccessful results following cycle sequencing. Instead of precipitating the samples following photometric quantification and dissolving them in a suitably low volume (which is a sure-fire recipe for sample loss), we recommend to re-concentrate the samples with the aid of Concentrator plus at a temperature of 60 °C using Function V-AQ. With these parameters, a DNA sample dissolved in water will constrict from 150 µl to 10 µl in a mere 30 minutes, without any adverse effect on the ability of the DNA to be sequenced. The same test conditions are selected for re-concentrating primers that are designated for use in sequencing. When sensitive samples (such as proteins) have to be re-concentrated, it is possible to use a lower temperature without any problems whatsoever, provided that the duration of concentration is extended accordingly.

2. Removing alcohol residues from the DNA sample

Contamination of the sample with traces of ethanol may cause sequencing reactions to fail. Alcohol residues that are present in the DNA samples after precipitation and washing of the pellets may be removed within four minutes by using Function V-AL at a temperature of 60 °C.

3. Aliquoting enzymes

Aliquoting a small amount of enzyme (e.g. RNase) into a variety of test tubes is far too time-consuming if each amount is weighed individually. To produce aliquots with 1 mg enzyme each, 100 mg enzyme is dissolved in 1 ml liquid (e.g. water) and aliquoted into 96 standard micro test tubes in quantities of 10 µl using an Eppendorf Multipipette plus. Each 10 µl enzyme solution completely evaporates within five minutes by using Function V-AQ at a temperature of 45 °C.

4. Using the Desiccator function

During the preparation of large amounts of plasmid DNA (maxi preparations), ethanol precipitation and centrifugation for DNA recovery is carried out in 25 ml or 50 ml tubes. If none of the special rotors offered for the Concentrator plus seems to be suitable for such large tubes, it is possible to remove ethanol and water residues using the Desiccator function. The test tubes are sealed using Parafilm®, the film is pierced and, after the rotor has been removed, the tubes are positioned in a stand (e.g. a polystyrene ring) and are transferred directly into the bowl. After the vacuum has been applied, the samples are incubated for 15 minutes at room temperature using Function D-AL. They are then free of alcohol residue and are dry to an extent that renders them ideal for re-suspending.

Conclusions

As shown by the examples mentioned above, the Concentrator plus is highly versatile and saves a great deal of time. It is able to meet the high throughput requirements of a professional sequencing laboratory thanks to the parallel sandwich use of two fixed-angle rotors, enabling preparation of up to 96 or 144 samples (in 1.5 ml or 0.5 ml tubes, respectively). Tool-free rotor exchange and operation without any additional rotor fastening means that even more invaluable time is saved.

A selection of different temperatures and various special functions guarantee flexible reaction conditions that are tailored to suit individual samples and solvents. Coupled with the high sample capacity, all of this combines to make Concentrator plus an indispensable assistant for our every-day routines.



Ordering Information Rotors

Description	Order No. International	Order No. North America
Rotor F-45-72-8 , for 72 Microcentrifuge tubes 0.5 ml	5490 034.007	022822080
Rotor F-45-70-11 , for 70 Microcentrifuge tubes 1.5/2.0 ml	5490 032.004	022822047
Rotor F-45-48-11 , for 48 Microcentrifuge tubes 1.5/2.0 ml	5490 030.001	022822004
Rotor A-2-VC , for 2 Microtest plates or 2 PCR plates	5490 045.009	022822241
Rotor F 45-24-12 , for 24 round bottom tubes of up to 6.0/8,0 ml (12 mm Ø x 67-100 mm in length)	5490 036.000	022822144
Rotor F-50-8-16 , for 8 round bottom tubes of up to 15.0/20,0 ml (16 mm Ø x 105 - 120 mm in length)	5490 041.003	022822233
Rotor F-50-8-18 , for 8 round bottom tubes of up to 15.0/20,0 ml (18 mm Ø x 105-128 mm in length)	5490 042.000	022822179
Rotor F-45-8-17 , for 8 Falcons® of up to 15 ml (17 mm Ø x 118 - 123 mm in length)	5490 038.002	022822225
Rotor F-40-36-12 , for 36 x 1.5 ml vials (12 mm Ø x 33 mm in length)	5490 040.007	022822209
Rotor F-45-36-15 , for 36 x 3.0/5.0 ml vials (15 mm Ø x 45 - 48 mm in length)	5490 035.003	022822128
Rotor F-45-16-20 , for 16 x 6.5/10.0 ml vials (20 mm Ø x 42-55 mm in length)	5490 043.006	022822136
Rotor F-40-18-19 , for 18 x 10.0 ml vials (19 mm Ø x 66 mm in length)	5490 037.006	022822161
Rotor F-45-12-31 , for 12 x 20.0 ml vials (31 mm Ø x 55 mm in length)	5490 044.002	022822217
Rotor F-35-8-24 , for 8 x 25.0 ml vials (24 mm Ø x 86 - 90 mm in length)	5490 039.009	022822187

Ordering Information Systems and Accessories

Article		Order No. International	Order No. North America
Concentrator plus (Vacufuge plus) , complete system with built-in diaphragm pump and 48 x 1.5/2.0 ml fixed-angle rotor, 50/60 Hz	230 V	5305 000.215	022820109
	120 V	5305 000.339	
Concentrator plus (Vacufuge plus) , complete system with built-in diaphragm pump, inclusive junction (e.g. for gel-dryers), without rotor, 50/60 Hz	230 V	5305 000.614	022820168
	120 V	5305 000.738	
Concentrator plus (Vacufuge plus) , complete system, with built-in diaphragm pump, without rotor	230 V	5305 000.410	—
	120 V	5305 000.533	
Concentrator plus (Vacufuge plus) , basic device (upgradable to complete system), with 48 x 1.5/ 2.0 ml fixed-angle rotor, 50/60 Hz	230 V	5305 000.010	022820001
	120 V	5305 000.134	
Spacer for sandwich use of fixed-angle rotors		5301 316.005	022822101
Adapter for 0.2 ml tubes for F-45-48-11, set of 6		5425 715.005	022636260
Adapter for 0.2 ml tubes for F-45-72-8, set of 6		5425 723.008	022636286
Adapter for 0.5 ml tubes for F-45-48-11, set of 6		5425 716.001	022636227
Work tray for 96 x 0.2 ml PCR tubes, set of 10		0030 124.235	951010031
Frame for work tray , set of 5		0030 124.243	951010049
CombiSlide® - Adapter, for the A-2-VC (set of 2)		5825 706.005	022638963

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