MILLIPORE

MultiScreen® Filter Plate with Ultracel®-10 Membrane: Nucleic Acid Applications

Application note: AN1040EN00

Introduction

The MultiScreen filter plate with Ultracel-10 membrane (MultiScreen Ultracel-10) is a 96-well filter plate designed for the purification, concentration and desalting of biological samples in the 0.1 to 0.5 mL range. MultiScreen Ultracel-10 incorporates a 10,000 NMWL (nominal molecular weight limit) regenerated cellulose membrane. It is compatible with standard microtiter plates, instrumentation and liquid handling equipment, making it highly suitable for high throughput applications.

MultiScreen Ultracel-10 is effective in the purification, concentration and desalting of protein solutions. This study will demonstrate further uses in the purification and concentration of single-stranded oligonucleotides, double-stranded DNA fragments, as well as plasmid and genomic DNA. Of particular importance, an examination of the effect of ionic strength on nucleic acid recovery revealed that the use of low salt buffer is essential for maximal yields.

Recovery of Oligonucleotides

The MultiScreen Ultracel-10 plate proves valuable for the recovery of very small DNA molecules such as single-stranded oligonucleotides (See Table 1). Yields of 55% for 10 or 20 mer oligos are impressive for such small molecules. It is important to note the effect of salt concentration on recovery as follows. Presentation of the sample in low salt results in higher yields across a range of oligo sizes. Recovery from high salt samples is much lower, with marked size-dependence. It is therefore recommended that for maximum recovery, small DNA species in particular be prepared in low salt buffer prior to MultiScreen Ultracel-10 filtration.

	Percent Recovery of Single-stranded Oligos			
	10 mer	20 mer	25 mer	48 mer
Low salt	55	55	63	52
High salt	15	20	33	50

Table 1. Recovery of 10 pmols of single-stranded oligonucleotides from 100 μL of low salt (10mM Tris pH 8.0) or high salt (10 mM Tris pH 8.0, 15 mM KCl, 1.5 mM $MgCl_2$) buffer after filtration at 2500 x g, for 60 minutes, n = 12. Filtration by vacuum is not recommended for oligoucleotides.

Recovery of Double-stranded DNA

MultiScreen Ultracel-10 is highly effective for the desalting and concentration of double-stranded DNA fragments. Consistent high yields of > 80% can be expected for fragments larger than 137bp, across a range of sample masses and volumes (See Table 2). As discussed previously, the recovery of samples presented in low salt buffer is significantly higher than for high salt samples. Dilution of samples prior to filtration is therefore recommended for optimal performance. This is especially true for small DNA fragments.

	Percent Recovery of Double-stranded DNA			
	0.1 ug challenge		1.0 ug challenge	
	100 uL	300 uL	100 uL	300 uL
137 bp	90	82	84	97
301 bp	96	90	90	89
657 bp	97	93	91	94
1159 bp	100	93	92	94

Table 2. Effect of sample mass and volume on recovery of double-stranded DNA fragments after vacuum filtration at 24" Hg (low salt buffer, n=6). Filtration by either centrifuge or vacuum results in similar, high recovery profiles. Recoveries measured by SYBR® Green assay (Molecular Probes).

Concentration of DNA

Concentration of DNA samples can be readily achieved using MultiScreen Ultracel-10 plates. This feature is invaluable in such downstream applications as sequencing, microarrays, or PCR amplification. In the example shown in Figure 1, a genomic DNA sample which was too dilute as a PCR template, was readily amplified after concentration using MultiScreen Ultracel-10.

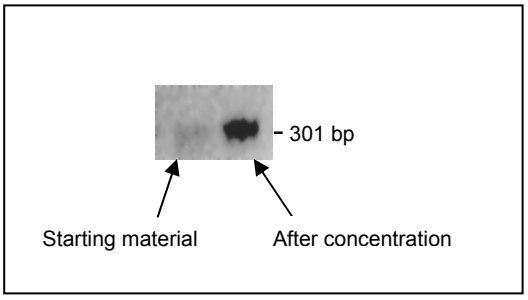


Figure 1. 1.5 % agarose gel showing use of MultiScreen Ultracel-10 for concentration of dilute genomic DNA samples enable successful PCR amplification. In this example, a sample of genomic DNA which had been concentrated 10-fold using MultiScreen Ultracel-10 was used as template in a PCR reaction specific to the human triglyceride lipase gene, a 301 bp fragment. The concentration step results in an enhanced band corrsponding to the gene of interest.

Desalting of DNA

Effective desalting or buffer exchange of nucleic acids is required for many downstream appications. The MultiScreen Ultracel-10 plate combines high recoveries with the ability to desalt and concentrate the DNA for up to 96 samples at a time. To demonstrate this feature, restriction digestion reactions, which are typically inhibited by high salt concentrations, can be successfully performed after purification of the samples using the MultiScreen Ultracel-10 plate (See Figure 2).

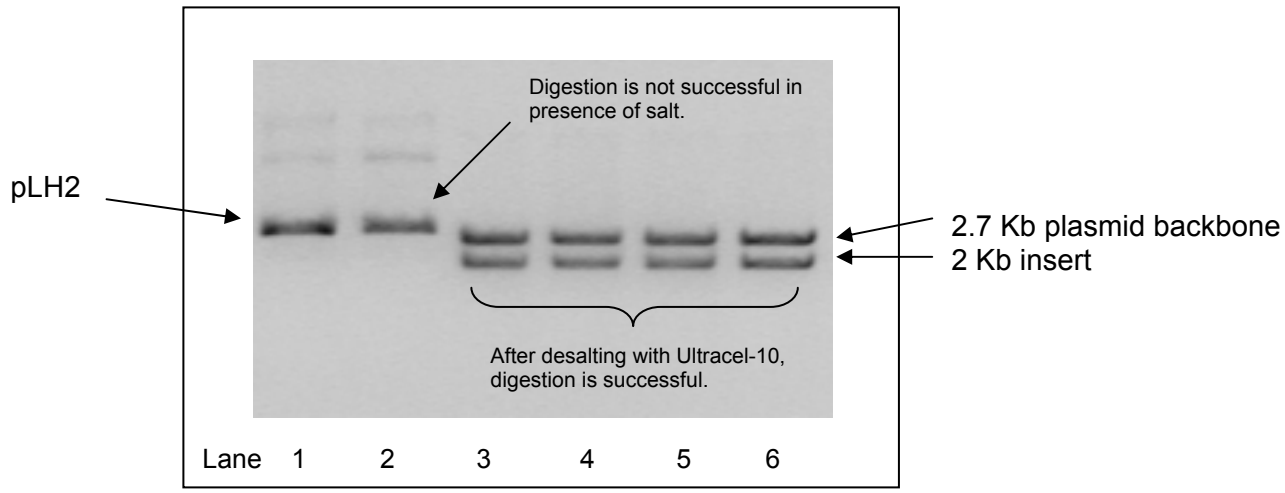


Figure 2. 1 % Agarose gel analysis showing successful restriction digestion after desalting using MultiScreen Ultracel-10. Lane 1: Undigested plasmid DNA (4.7 kb) in high salt (10 mM Tris pH 8.0, 15 mM KCl, 1.5 mM $MgCl_2$). The arrow represents the uncut, supercoiled form.
Lane 2: Result after digestion of a 1 μg sample using I unit of Hind III (New England Biolabs) at 37ºC for 1 hour. The high salt inhibits the digestion resulting once more in only one band.
Lanes 3-6: The removal of salt by MultiScreen Ultracel-10 allows digestion to occur resulting in two distinct bands.

MultiScreen Ultracel-10 Features

Flow Rates

Typical flow times for Ultracel plates using both vacuum and centrifuge are shown in Table 3.

	24" Hg	2500 x g
100 µL	35 minutes	70 minutes
300 µL	75 minutes	90 minutes

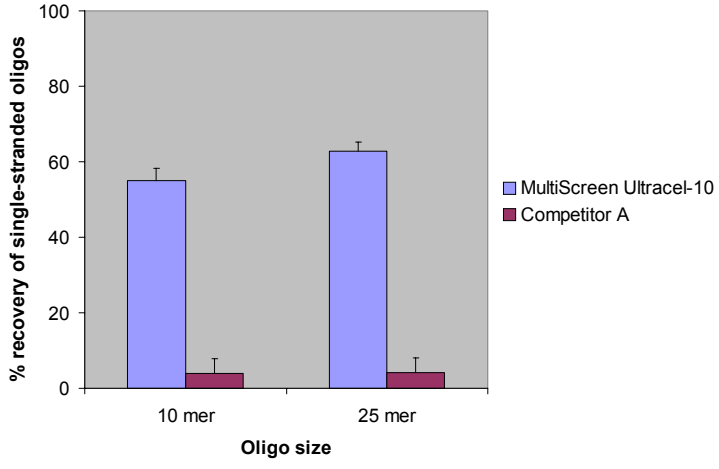
Table 3: MultiScreen Ultracel-10 flow times for vacuum and centrifugal filtration with 100 and 300 µL volume challenges. These values are for guidance only as flow rate will depend on DNA fragment size, as well as challenge mass and volume.

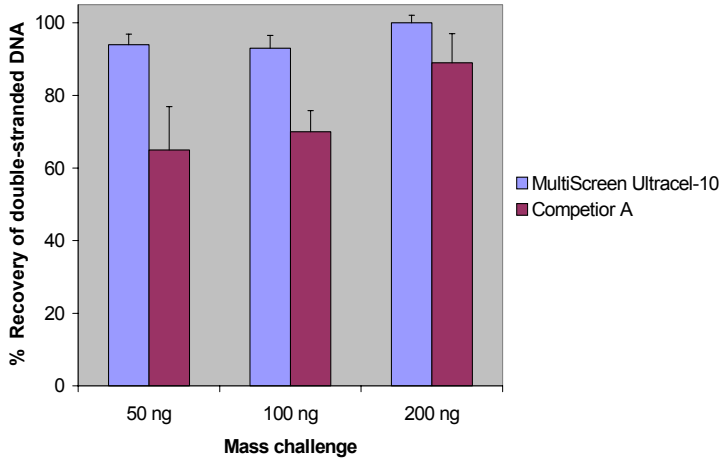
Use of Partial Plates

In situations where only a small number of samples are being purified, use of only a small number of wells of the MultiScreen Ultracel-10 plate is acceptable. Unused wells can be employed for a subsequent experiments without impairment of performance.

Competitive Evaluation

MultiScreen Ultracel-10 outperforms its major competitor in a variety of DNA applications as shown by the examples in Tables 3A and 3B below. MultiScreen Ultracel-10 plates allow significantly higher recoveries of both single and double- stranded DNA for both vacuum filtration and centrifugation. This reflects the tighter membrane structure of MultiScreen Ultracel-10 and represents a major advantage to





the end user.

Figure 3A. Recovery of 10 pmol of 10 mer and 25 mer oligos from low salt (TE pH 8.0) buffer after centrifugation at 2500xg using MultiScreen Ultracel-10 or Competitor A plates.

Figure 3B. Recovery of 50, 100, and 200 ng of 137 bp DNA from low salt (TE pH 8.0) buffer after vacuum filtration at 24" Hg using MultiScreen Ultracel-10 or Competitor A plates.

Summary

The MultiScreen Ultracel-10 plate is a valuable addition to the Millipore range of 96 well filtration plates, and is recommended not only for protein applications but also where purification, concentration and desalting of DNA samples is required. Due to the tight nature of the membrane, high recoveries of both single-stranded and double-stranded DNA can be achieved, especially from samples presented in low salt buffer. Recovery of larger DNA fragments such as plasmid and genomic DNA is successful regardless of ionic strength. Ultracel-10 is compatible with most liquid handling systems making high throughput a further advantageous feature of the plate.

Appendix

Generalized protocol for MultiScreen Ultracel-10

Apply samples to the MultiScreen Ultracel-10 plate.
Align with receiver plate (centrifugal filtration only).
Filter by vacuum at 24" Hg or by centrifugation at 2500 x g to dryness.
Add resuspension solution and shake on a plate shaker at maximum speed for 10 minutes.
Remove collected sample to a suitable storage plate.

SYBR Green assay

Dilute samples 1 in 100 and mix with an equal volume of Sybr Green I nucleic acid stain (1 in 6000).
Measure fluorescence at 480/535 nm and compare with a standard curve.

Related information

MultiScreen Ultracel-10 data sheet: PF2050EN00