High Pure PCR Cleanup Micro Kit – Novel Flexibility for Nucleic Acid Purification

*Nina Lassonczyk, Constanze Stadler, Jenna Liedtke, and Horst Donner**
Roche Applied Sience, Penzberg, Germany
**Corresponding author: horst.donner@roche.com*

Manual nucleic acid isolation with High Pure products is long approved by the scientific community. The product portfolio includes kits for isolation of genomic RNA and DNA, plasmid DNA as well as purification of reaction products. Key goal for all products is to offer optimal solutions suited for many different sample materials in order to reduce the number of different nucleic acid isolation kits necessary in modern molecular biology labs.

Introduction

The High Pure Micro format is a new format introduced last year with the High Pure FFPE RNA Micro Kit. This format enables researchers to isolate and purify nucleic acids in micro scale with low elution volume (>10 µl) and at high concentrations in order to avoid time consuming and sample endangering concentration steps.

High Pure PCR Cleanup Micro Kit Description

The High Pure PCR Cleanup Micro Kit extents this product line by an additional application routinely used in nearly all molecular biology workflows. PCR products as well as nucleic acids derived from enzymatic polymerization, labeling, or modification can be purified in micro scale. The High Pure PCR Cleanup Micro Kit is based on the well established spin column approach including binding, washing, and elution steps for purification. This principle is fast, easy, convenient, and well established for more than 10 years.

Whereas some commercial suppliers offer different kits for purification of PCR products, labeling reactions, or purification from agarose gel slices, the High Pure PCR Cleanup Micro Kit is designed to address all of these different sample materials in one kit. Two protocols are included: one for the purification of up to 100 µl liquid sample and one for up to 100 mg agarose gel slices.

A new level of flexibility is achieved by providing a binding buffer and a binding enhancer solution separately. Both components can be mixed individually in order to adjust the stringency and binding properties. The amount of binding enhancer added directly correlates with the affinity of the binding process on the column matrix. Therefore yield and size exclusion is variable according to the amount of binding enhancer used. The importance of these parameters varies in different applications and recommended values are shown Table 1. For purification of labeling reaction products or PCR products without the need of primer-dimer depletion, the addition of 200 µl binding enhancer is recommended to achieve maximal stringency (option 1). For the removal of small oligonucleotides like simple primers a mixture of 300 µl binding buffer and 100 µl binding enhacer is optimal in respect to purity and yield of the purified nucleic acid (option 2). For purification of PCR products for sequencing reactions, omittance of binding enhancer guarantees freedom from any contaminating primer or primer-dimer artifacts, which could negatively impact sequencing results even in small traces. Therefore maximal depletion of

Table 1: Application selection guide.

For 100 µl liquid sample or 100 mg agarose gel		**Option 1** 200 µl binding buffer + 200 µl binding enhancer (40%)	**Option 2** 300 µl binding buffer + 100 µl binding enhancer (20%)	**Option 3** 400 µl binding buffer
Purification of liquid sample, 100 µl	Labeling or other reaction products 100 bp to 5 kb	+		
	PCR products 100 bp to 5 kb	+		
	DNA fragments for sequencing			+
Purification from agarose gel, 100 mg	DNA Fragments 100 bp to 5 kb		+	
Removal of low molecular weight DNA	Primer up to 25 bases		+	
	Primer-dimer up to 70 bp			+

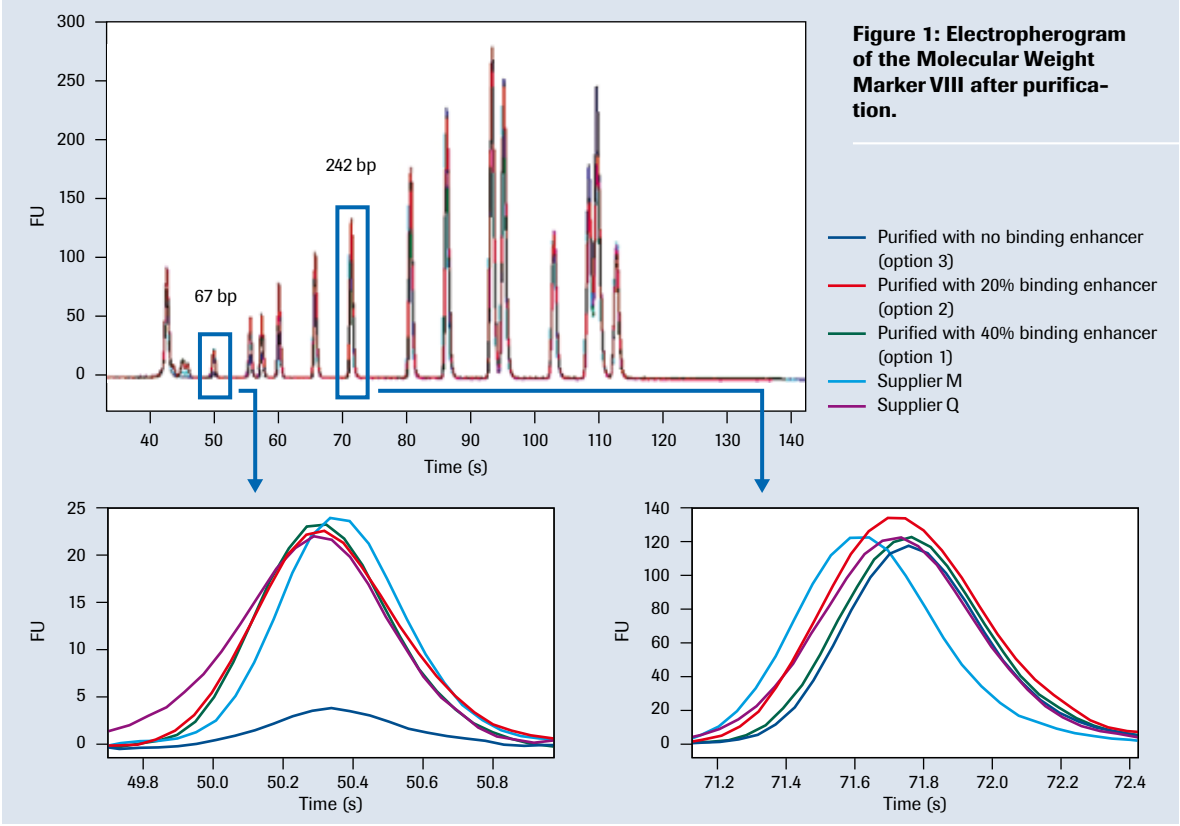

Figure 1: Electropherogram of the Molecular Weight Marker VIII after purification.

low molecular nucleic acids (*i.e,* primers and primer-dimers) is observed when no binding enhancer is added to the sample – binding buffer mixture (option 3).

Application Examples

In order to demonstrate the performance of the High Pure PCR Cleanup Micro Kit, 3 µg of Molecular Weight Marker VIII (including dsDNA fragments from 19 bp to 1114 bp) were mixed with Fast Start HiFi PCR reaction buffer and purified with the High Pure PCR Cleanup Kit, using increasing amounts of binding enhancer. For further analysis equal amounts of each sample were subjected to a capillary gel electrophoresis utilizing the DNA 1000 chip on a Bioanalyzer instrument (Agilent, USA).

Figure 1 displays the result obtained from a Bioanalyzer analysis of Molecular Weight Marker VIII after purification with the High Pure PCR Cleanup Kit using increasing amounts of binding enhancer as well as two other commercial available kits. Only the purification with the High Pure PCR Cleanup Kit without addition of binding enhancer shows a good depletion of the 67-bp-fragment. The analysis of the 242-bp-fragment shows a similar representation of this DNA fragment in all samples. The impact of size exclusion and depletion of small nucleic acid molecules is especially of importance when PCR products are purified. Two synthetic DNA molecules are required for each PCR and serve as primer for the synthesis reaction. Although reaction conditions could be optimized in respect to yield and specificity most of the PCR reactions still contain remaining primer and to a variable amount primer-dimer artifacts.

For test purposes, a 341-bp PCR fragment of the tPA gene was amplified using Taq DNA Polymerase, a $MgCl_2$ containing reaction buffer, and a standard protocol for block-cycler instruments. The resulting reaction mixes were pooled and purified using variable amounts of binding enhancer and analyzed on a NanoDrop instrument by OD measurement. For further analysis 250 ng of the purified PCR products were subjected to a 1% agarose gel electrophoresis. Binding enhancer in the isolation procedure increases the recovery of DNA fragments from the PCR product mix. This increase mainly affects lower molecular PCR fragments (like primer-dimers). The yield obtained from the PCR reactions displayed in Figure 2 is shown in Table 2.

Increasing amounts of binding enhancer lead to an increasing recovery of DNA fragments from the PCR reaction mixture. Nevertheless as shown in Figure 2, a substantial amount of this increase in yield is based on the higher amount of primer-dimers co-purified from the PCR reaction mixture. The impact of remaining PCR artifacts for down-stream applications was demonstrated by using similar amounts of purified PCR fragments as templates for a re-PCR on a LightCycler® 1.5 Instrument. The reaction was carried out in duplicates including primers used for the block cycler PCR

(Figure 3). The negative control (PCR without template DNA) shows a high primer-dimer peak as well as no PCR product peak of the tPA gene fragment. Using PCR fragments purified with increasing amounts of Binding Enhancer as template, an increasing amount of primer-dimers together with decreasing amounts of tPA gene specific PCR product peaks are observed. This effect is based on the increasing co-purification of primer-dimers from the first PCR which served as template in the light cycler PCR.

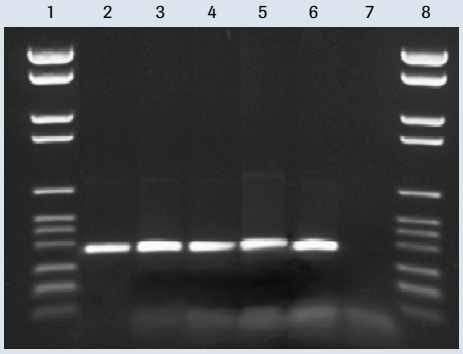

Figure 2: Agarose gel electrophoresis and ethidiubromide staining of 341 bp PCR products.

Table 2: Yield of PCR fragments isolated with increasing amounts of binding enhancer.

Binding conditions	260/280 ratio	Yield (µg)
No binding enhancer (option 3)	1.9	0.6
10% binding enhancer	1.9	0.9
20% binding enhancer (option 2)	1.8	1.2
40% binding enhancer (option 1)	1.8	1.2

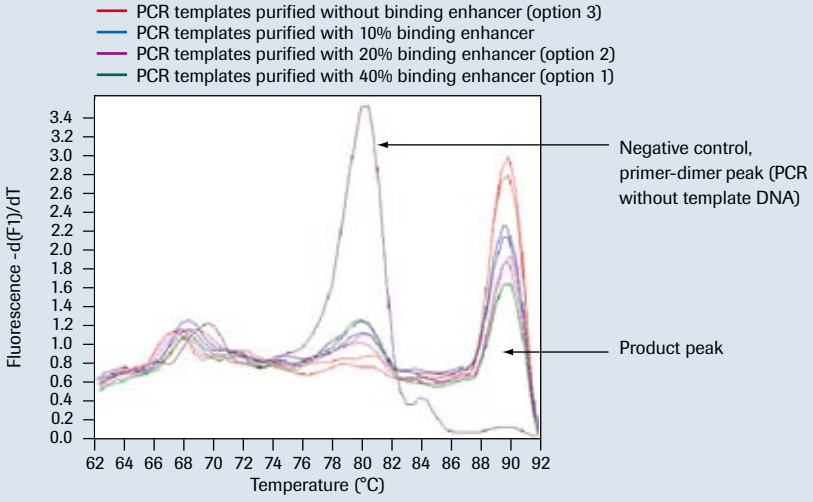

Figure 3: Melting curve analysis after the last PCR cycle using a LightCycler® 1.5 Instrument.

Table 3: Purification of an 1,100 bp cDNA fragment in combination with depletion of Cy5 dUTP.

Sample ID	Yield (µg)	260/280 ratio	Cy5 dye (mmol)
Supplier Q	0.1	1.9	not detectable
Supplier M	1.1	2	0.22
High Pure PCR Cleanup Micro Kit + 20% binding enhancer (option 2)	1.8	2.1	not detectable
High Pure PCR Cleanup Micro Kit + 40% binding enhancer (option 1)	1.9	2.1	0.01

For many other applications size exclusion is of minor importance compared to high yield and absence of reaction components like enzymes, unincorporated nucleotides and labels. In order to evaluate these features, one microgram of an 1,100-b mRNA fragment was reverse transcribed utilizing the Microarray cDNA Labeling Kit. After reverse transcription, the synthesis reaction was stopped by adding EDTA. For the measurement of the depletion of labeled nucleotides 2.2 nmol of Cy5 dUTP per purification was added. Reaction products were immediately purified using the High Pure PCR Cleanup Micro Kit with 20% and 40% Binding Enhancer (option 2 and 1) according to the kit manual. Results were compared with those obtained by two commercially available products. Table 3 displays the results from purifications of reverse transcription products in the presence of Cy5 dUTP. The High Pure PCR Cleanup Kit shows the highest yield compared with both commercial purification products. Also the amount of remaining Cy5 dUTP is below (20% binding enhancer) or at the end of the detection limit (40% binding enhancer) of the NanoDrop instrument.

Conclusions

The elution volume of down to 10 µl together with a binding capacity of up to 20 µg DNA per purification fits well to experimental needs. The flexibility of adjusting the binding efficiency by providing a universal binding buffer and a binding enhancer separately enables researchers to adjust the amount of binding enhancer used and therefore to vary the size exclusion of the purification process. Primer-dimers and low molecular DNA fragments are efficiently removed by purification without binding enhancer. In contrast, the use of up to 40% binding enhancer in the binding solution reveals high yields as well as high purity shown by the depletion of Cy5 dUTP from a cDNA synthesis reaction. This flexible kit concept enables researchers to specifically address their needs without using different kits, purification principles, or workflows. The good performance and the broad range of applications makes the High Pure PCR Cleanup Micro Kit a universal tool for most purification issues in modern molecular biology labs.

Order INFO

Product	Pack Size	Cat. No.
High Pure PCR Cleanup Micro Kit	50 purifications	04 983 955 001
	200 purifications	04 983 912 001