# RapidClean™

## Completely remove protein from nucleic acid samples in less than 5 minutes

**Many molecular biology procedures involve the amplification or modification of nucleic acids using enzymes that must then be removed or heat-inactivated before proceeding with subsequent steps. RapidClean is a novel protein-binding resin that allows all the proteins in an aqueous sample to be collected with the resin in a pellet via centrifugation. The entire procedure takes under 5 minutes, saving time and avoiding the inconvenience of heat inactivation or phenol-cholorform extraction.**

RapidClean is a solid-phase resin that removes enzymes and other proteins from aqueous solution. In less than five minutes, nucleic acid samples are protein-free and ready to use in the next step of a procedure. RapidClean resin is suitable for single stranded and double stranded nucleic acids, from five bases to greater than 40 kb in size, including DNA, RNA and oligonucleotides. Recovery rates exceed 95%.

## Results comparable to phenol-chloroform extraction

### Plasmid purification

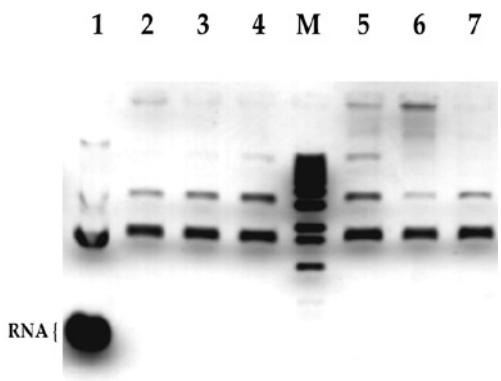
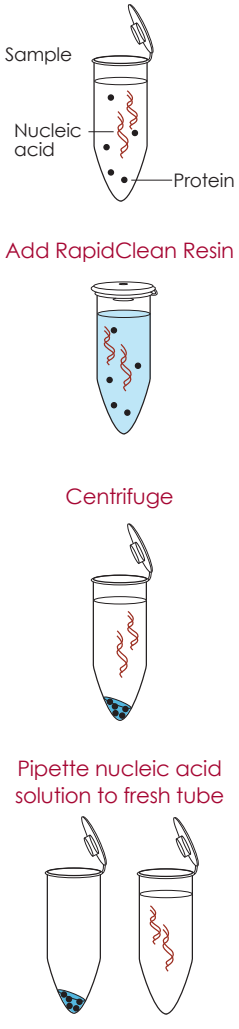



**Figure 1. RapidClean purifies pUC119 plasmid as efficiently as phenol-chloroform extraction.** NaOH/SDS-treated and potassium acetate neutralized lysate was spun down at 12,000 xG for 5 min and precipitated with 2 volumes of ethanol. Crude plasmid pellet was resuspended in TE buffer (lane 1), treated with RNase (lanes 2,5) and extracted with RapidClean one time (lane 3) or twice (lane 4) or phenol-chloroform extracted once (lane 6) or twice (lane 7). Lane M: molecular weight marker.

RapidClean provides a quick, non-toxic alternative to phenol-chloroform extraction when cleaning up nucleic acid samples, such as in plasmid purification procedures.

Phenol-chloroform extraction is inconvenient, requiring the use of toxic reagents and the production and disposal of organic waste. Additionally, organic extractions can be associated with sample loss, due to incomplete pipetting of the aqueous phase, and disturbance of the phase interface during pipetting can result in carryover protein contamination. The requirement for ethanol precipitation to remove any residual phenol can result in additional sample loss, and if the starting concentration of nucleic acid is low, or the size of the nucleic acid is small, ethanol precipitation will be especially time consuming and inefficient.

**RapidClean Resin Protocol**



RapidClean has no requirement for nucleic acid type, size, or concentration. Low concentration and low molecular weight nucleic acids can be cleaned with recovery rates greater than 95% in less than 5 minutes. In purification of a pUC119 plasmid prep (3162 bp), extraction with RapidClean provides results comparable to extraction with phenol-chloroform (Figure 1).

## Speed up your protocols

### Preventing PCR carryover

To prevent PCR carryover or laboratory contamination by PCR products, uracil is often incorporated into PCR reaction mixes, and uracil DNA glycosylase (UDG) included to hydrolyze any pre-existing PCR product before amplification begins. After the initial hydrolysis step, the UDG must be inactivated, usually via heating. However, even after heat inactivation, approximately 5% of UDG activity can remain, leading manufacturers to suggest phenol-chloroform extraction to remove residual UDG, or inclusion of a UDG inhibitor in following steps, to improve yields.

RapidClean avoids the necessity for heat inactivation and/or phenol cholorform extraction, completely removing UDG from a reaction mix in two quick extractions (Figure 2).

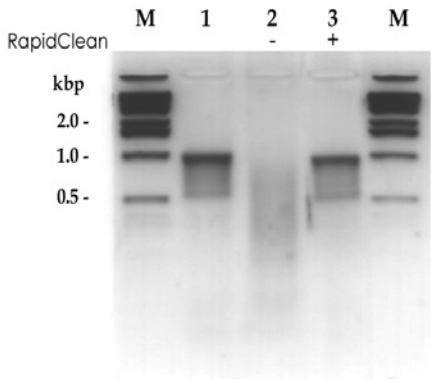



**Figure 2 RapidClean removes uracil DNA glycosylase.** 1 unit of UDG (Boehringer) in 20 µl of 1x GeneAmp Buffer (Perkin Elmer) was extracted 2 times with 2.0 µl of RapidClean each, followed by the addition of 10 µl of PCR mix containing a1kb double-stranded dU-DNA fragment as substrate, and incubation at 95 °C for 7 min. Lane M: molecular weight marker. Lane 1: dU DNA fragment heated to 95 °C for 7 minutes. Lane 2: dU DNA fragment heated to 95 °C for 7 minutes with complete UDG reaction mix. Lane 3: dU DNA fragment heated at 95 °C for 7 minutes with UDG reaction mix extracted twice with RapidClean to remove UDG.

## No Molecular Weight Limitations

### Remove enzymes from oligonucleotides

Other nucleic acid clean up methods, such as spin columns, are limited to use with nucleic acids longer than 70 bp. RapidClean effectively removes protein from solutions of nucleotides ranging in size from 5 nucleotides to 40 kb. Figure 3 demonstrates the removal of T3 RNA polymerase, MuLV Reverse transcriptase and RNase H from a 58-mer oligonucleotide.

Note that RapidClean releases the oligonucleotide from protein-nucleic acid complexes, allowing the free oligonucleotide to enter the gel.

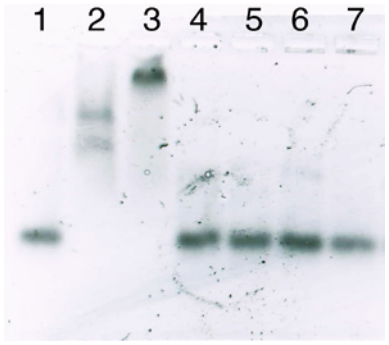



**Figure 3. RapidClean removes protein from oligonucleotides.** A 58mer oligonucleotide (lane 1) was incubated with T3 RNA polymerase (lanes 2,5), MuLV reverse transcriptase (lanes 3,6) or RNase H (lanes 4,7) either with (lanes 5,6,7) or without (lane 2,3,4) extraction with RapidClean. In each case, extraction with RapidClean removed the enzymes and released the oligo from the oligo-enzyme complex.

## Global Protein Removal

### Remove nucleases, polymerases, ligases

RapidClean resin binds all proteins, so it can be used in any situation to remove any enzyme or protein from a reaction mix. Figures 4 through 6 demonstrate the use of RapidClean to remove nucleases, Taq DNA polymerase, and T4 Ligase from reactions.

Long heat inactivation steps (Exonuclease III) and hazardous phenol-chloroform extractions (Mung Bean Nuclease), can be avoided since RapidClean can remove these enzymes quickly and safely.

## Other applications

For any application that requires deproteinization, consider using RapidClean.

Removing protein from DNA and RNA samples prior to electrophoresis can improve band resolution by eliminating protein-nucleic acid interactions that interfere with migration into the gel. With RapidClean, deproteinization of gel samples is quick and easy.

RapidClean can also be used to concentrate proteins from dilute solutions for electrophoresis or Western blotting (Application Note 102).

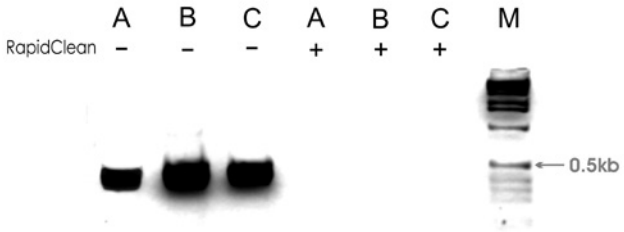



**Figure 5. RapidClean completely removes DNA polymerase.** No Taq DNA Polymerase activity remains after extraction with RapidClean. PCR reactions using polymerase from three different vendors (A, B, C) were extracted (+) or not extracted using RapdClean, and then subjected to 30 PCR cycles. No amplified product was detected in the samples extracted with RapidClean.

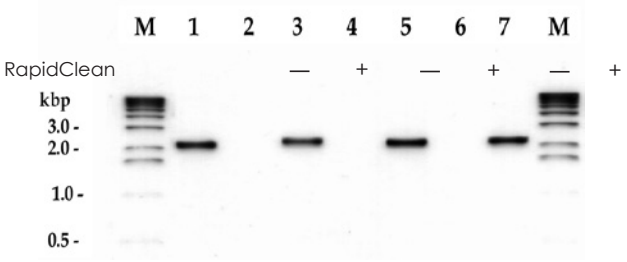



**Figure 4. RapidClean removes DNase I, Mung bean nuclease, and S1 nuclease.** M12mp18 ssDNA (lane 1) is completely digested by 30 minute incubations at 37°C with DNase 1 (lane 2), Mung Bean Nuclease (lane 4) or S1 nuclease (lane 6), but no residual nicking activity is observed if the reactions are first extracted twice with RapidClean (lanes 3, 5, and 7). Lane M: 1 kb DNA ladder.

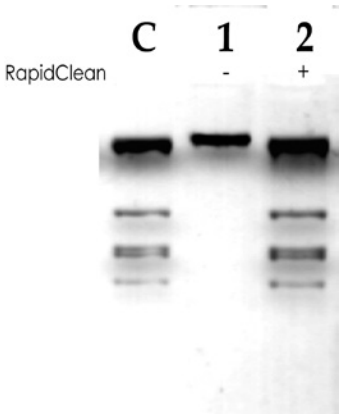



**Figure 6. RapidClean™ completely removes T4 DNA ligase.** No detectable ligase activity remains in a reaction mix after extraction with RapidClean™ (lane 2). EcoR I digested λ-DNA (lane C, Control) was incubated for 16 hours at room temperature with T4 DNA ligase (lane 1) or with the same mixture extracted with RapidClean™ (lane 2).



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