

HEAT-LABILE ENZYMES

Specific, High Activity
Enzymes

Completely Inactivated
by Heat

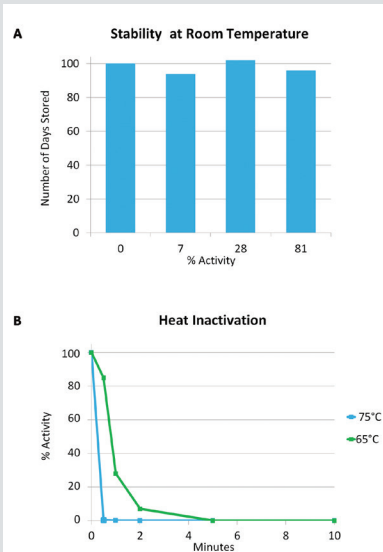
No Enzymatic
Carry Over

Shrimp Alkaline Phosphatase, Recombinant (rSAP)

Shrimp Alkaline Phosphatase, Recombinant (rSAP) is a heat-labile hydrolase enzyme produced in *Pichiapastoris* that removes phosphate groups nonspecifically from 5' ends of nucleic acid phosphomonoesters and proteins. This activity is most commonly utilized in molecular cloning to prevent self-ligation of linearized plasmid DNA and in 5' end-labeling to facilitate the replacement of unlabeled phosphates with labeled phosphate groups. rSAP also prepares PCR products for DNA sequencing or SNP analysis, by dephosphorylating unincorporated dNTPs that would otherwise interfere with enzymatic reactions.

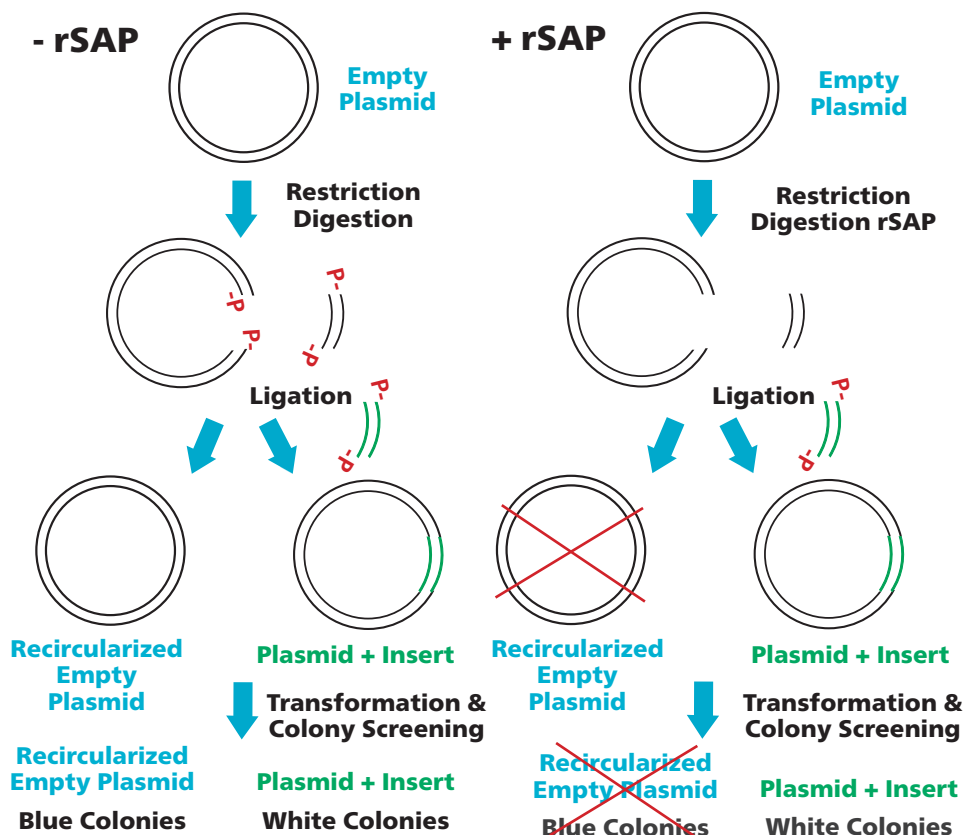
VWR AMRESCO's rSAP may be directly added to restriction enzyme digests and is conveniently 100% inactivated by heating at 65°C (149°F). This eliminates the need for vector purification, a necessary step when using alkaline phosphatases isolated from other sources, such as *E. coli* and calf intestine. rSAP works well in common buffers and does not require supplemental zinc or other additives.

- Removes 5'-phosphates from DNA, RNA, dNTPs, and proteins
- Improves cloning efficiency by preventing vector recircularization
- 100% heat-inactivated at 65°C (149°F)
- No vector purification necessary
- Removes unincorporated dNTPs in PCR products prior to DNA sequencing or SNP analysis
- Prepares templates for 5' end labeling
- Dephosphorylates proteins
- Works in many different buffers without supplemental factors

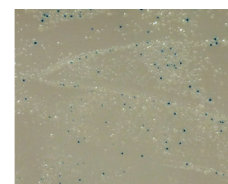
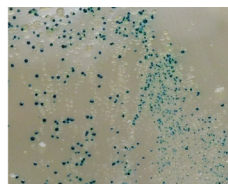


(A) rSAP was tested for activity at several time points after up to 81 days of storage at room temperature. Even with prolonged room temperature storage, rSAP maintained 96% of its original activity. (B) Samples of rSAP were heated to 75°C or 65°C for up to 10 minutes, with activity measured at several time points. After just 2 minutes at 75°C and 5 minutes at 65°C, rSAP activity declined to 0%.

Size	Cat. No.	Unit
1KU	10063-736	Each
5KU	10063-738	Each



Shrimp Alkaline Phosphatase, Recombinant (rSAP) treatment reduces empty vector background in molecular cloning. Linearized pUC19 was treated with rSAP or water prior to ligation with an insert. The rSAP was irreversibly heat-inactivated and the samples were used without any purification steps before the ligation reaction.

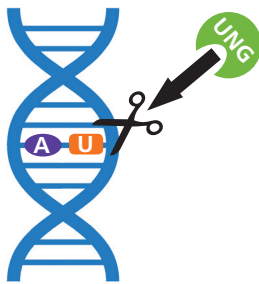


PCR Mix

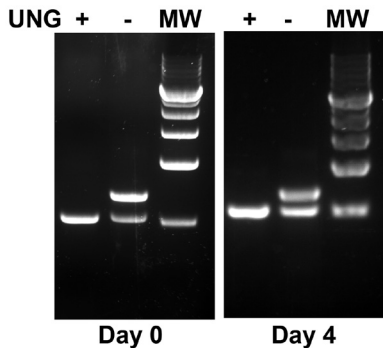


Taq DNA polymerase
Reaction buffer
Primers
dNTPs (with dUTP)
DNA template (dTTP)
UNG

↓ 5 min @ RT



↓ 5 min @ RT



Uracil-DNA Glycosylase (UNG), Cod treatment specifically degrades dUTP-containing DNA in spiked PCR reactions and inactivates completely to allow subsequent synthesis of new dUTP-containing amplicon. Two PCR reactions were prepared with normal dTTP-containing DNA template and then spiked with a dUTP-containing amplicon from a previous PCR reaction. One PCR reaction was treated with UNG (10063-740) for 5 minutes at room temperature, while the other tube was left untreated. The treated and untreated samples were then immediately amplified by PCR, with the initial denaturation cycle serving as the heat inactivation step for the UNG treated sample. Following amplification, the PCR products with incorporated dUTP were analyzed by gel. The amplicons were stored at room temperature for 4 days and then analyzed by gel again. In the UNG treated sample, only one band was present due to effective degradation of the dUTP DNA. The dUTP DNA synthesized from dTTP template after UNG heat inactivation remained stable, even when stored at room temperature.

Uracil-DNA Glycosylase (UNG), Cod

Uracil-DNA Glycosylase (UNG), Cod is a thermolabile recombinant enzyme produced in *E. coli* (ung-) using a modified ung gene derived from Atlantic Cod. It degrades uracil-containing single- and double-stranded DNA, but not RNA or thymidine-containing DNA, by hydrolyzing the N-glycosidic bond between deoxyribose sugar and the base in uracil. This generates alkaline-sensitive apyrimidinic sites in the DNA that will be cleaved upon a combination of alkaline conditions and high temperature.

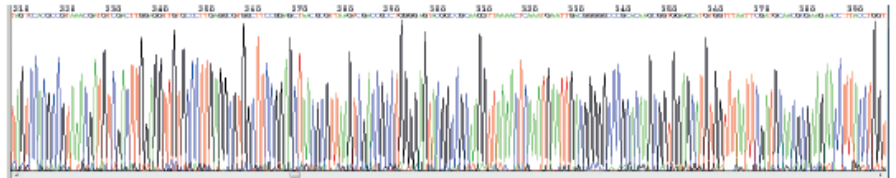
Pretreatment of samples with UNG prevents PCR carryover contamination in labs that substitute dUTP in place of dTTP during all amplification reactions. PCR products containing uracil become substrates for UNG and will be degraded if they are present in subsequent reaction mixtures subjected to UNG treatment. Only DNA templates containing thymidine are not degraded by the treatment and will be amplified.

Recombinant cod UNG is irreversibly heat inactivated, which enables long-term storage and subsequent analysis of post-PCR amplicons in applications such as cloning and sequencing. VWR AMRESCO's UNG is compatible with PCR, qPCR and one-step qRT-PCR and works in all commercially available master mixes. All amplification reactions must use dUTP containing dNTP mixtures in order for the UNG decontamination method to be effective.

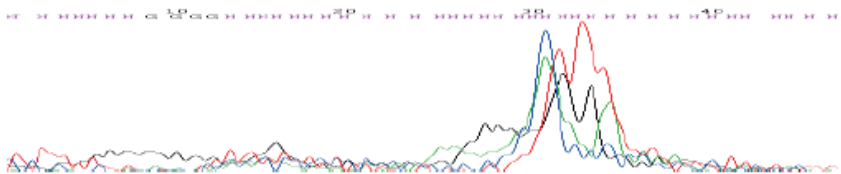
- Complete, irreversible heat-inactivation
- Prevents carryover contamination in PCR, qPCR and qRT-PCR
- Effective with only a 5 minute incubation step

Size	Cat. No.	Unit
100U	10063-740	Each
1000U	10063-742	Each

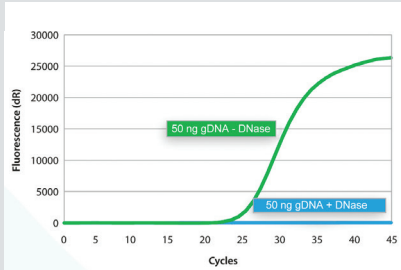
A



B



VWR AMRESCO Uracil-DNA Glycosylase (UNG), Cod is completely inactivated by heat, enabling newly synthesis of new dUTP-containing amplicons that remain stable for downstream sequencing. Prior to amplification, two PCR reactions were treated with either UNG (A) or *E. coli* UNG(B). The chromatograms reveal that only the PCR product from the reaction treated with VWR AMRESCO UNG gave a readable sequence.

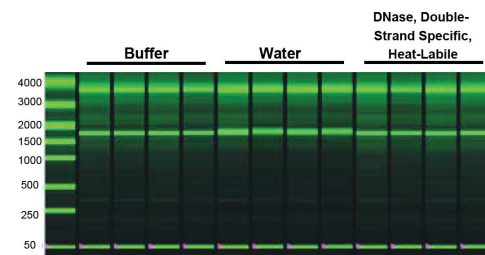


Decontamination of a PCR master mix using Heat-Labile DNase, Double-Strand Specific. A PCR master mix containing 50ng genomic DNA was prepared and divided into two reactions, of which one was treated with HL-dsDNase prior to qPCR of both samples. In contrast to the untreated sample, the DNase-treated sample contained no amplifiable genomic DNA.

Heat-Labile DNase, Double-Strand Specific (HL-dsDNase)

VWR AMRESCO's Heat-Labile, Double-Strand Specific DNAase is a recombinant endonuclease that cleaves phosphodiester bonds in DNA to yield 2–8bp oligonucleotides with 5'-phosphate and 3'-hydroxyl termini. The high specific activity of this enzyme toward dsDNA can be inactivated by heating at 55°C (131°F), conveniently eliminating the need for its physical or chemical removal before downstream processing, even in the presence of RNA and ssDNA, such as primers and probes. HL-dsDNase is ideal for removal of genomic DNA in RNA preps and for removal of DNA carryover contamination in PCR mixes, before the addition of the template.

- High specificity for dsDNA, while leaving ssDNA and RNA intact
- Heat inactivates completely and irreversibly at 55°C (131°F)
- Ideal for genomic DNA removal in RNA preps



Heat-Labile DNase, Double-Strand Specific treatment leaves RNA quality intact. RNA incubated with buffer, water, or DNase were analyzed using the Eukaryote Total RNA StdSens Assay, with the results indicating all samples had intact, high quality RNA (RQI > 8.5).

Size	Cat. No.	Unit
250U	10147-168	Each
1000U	10147-174	Each

Supporting Products Often Used with VWR Life Science AMRESCO Heat-Labile Enzymes

Description	Size	Cat. No.
PCR Reagents		
TAQ DNA Polymerase	500 U	97064-160
Electrophoresis		
Agarose I™	25 g	97062-248
Agarose I™	100 g	97062-244
Agarose I™	500 g	97062-250
Agarose SFR™	25 g	97064-138
Agarose SFR™	100 g	97064-134
EZ-Vision® One DNA Dye as Loading Buffer, 6X	5 x 1 mL	97064-190
EZ-Vision® Two DNA Dye as Loading Buffer, 6X	5 x 1 mL	97063-268
EZ-Vision® Three DNA Dye as Loading Buffer, 6X	5 x 1 mL	97063-166
TAE, Liquid Concentrate, 25X	1.6 L	97062-384
TAE, Liquid Concentrate, 25X	4 L	97062-386
TBE, Liquid Concentrate, 5X	1 L	97063-432
TBE, Liquid Concentrate, 5X	4 L	97063-436

Description	Size	Cat. No.
Microbiological Media and Selective Agents		
Ampicillin, Sodium Salt	25 g	97061-442
Ampicillin, Sodium Salt	100 g	97061-440
Freezing Media, Bacterial	25 Packs	97063-178
Kanamycin Sulfate	10 g	97061-600
Kanamycin Sulfate	25 g	97061-602
Kanamycin Sulfate	100 g	97061-598
LB Agar, Miller Formulation	1 kg	97064-106
LB Broth, Miller (Luria-Bertani)	500 g	97064-114
LB Broth, Miller (Luria-Bertani)	1 kg	97064-110
LB Broth, Liquid	1 L	89500-596



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