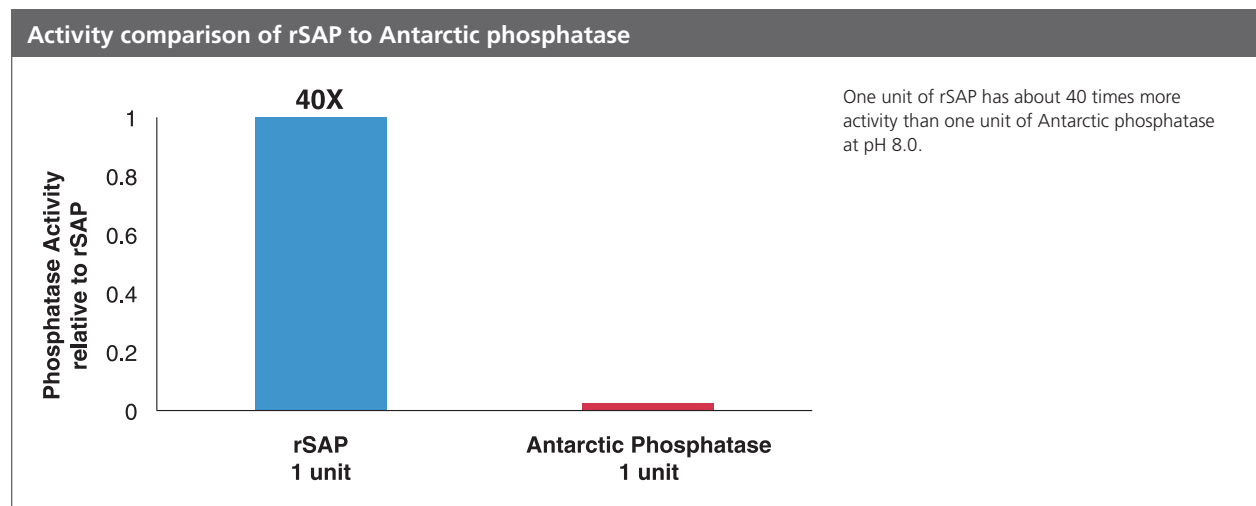


Frequently asked questions

USB® Recombinant Shrimp Alkaline Phosphatase (rSAP), [PN 78390]

Which alkaline phosphatase works best?

Recombinant SAP (rSAP) is preferred over calf intestinal alkaline phosphatase (CIAP) because it is 100% heat-inactivated after incubation for 5 minutes at 65°C. rSAP is preferred over Antarctic phosphatase because it requires no buffer modifications for activity. Also, Antarctic phosphatase is 40-fold less active than rSAP in the pH range of most enzyme reaction buffers (pH 7.5-8.5).



What phosphate groups are removed by rSAP?

rSAP removes phosphates from the 5' ends of single-stranded and double-stranded DNA and RNA. rSAP also removes 3'- and 2'- monophosphates from DNA. Protein dephosphorylation using shrimp alkaline phosphatase has also been reported^(1,4).

How long does rSAP retain 100% activity in its storage buffer at various temperatures?

Temperature	-20°C	4°C	25°C
100% activity	> 1 year	> 4 months	> 2 months

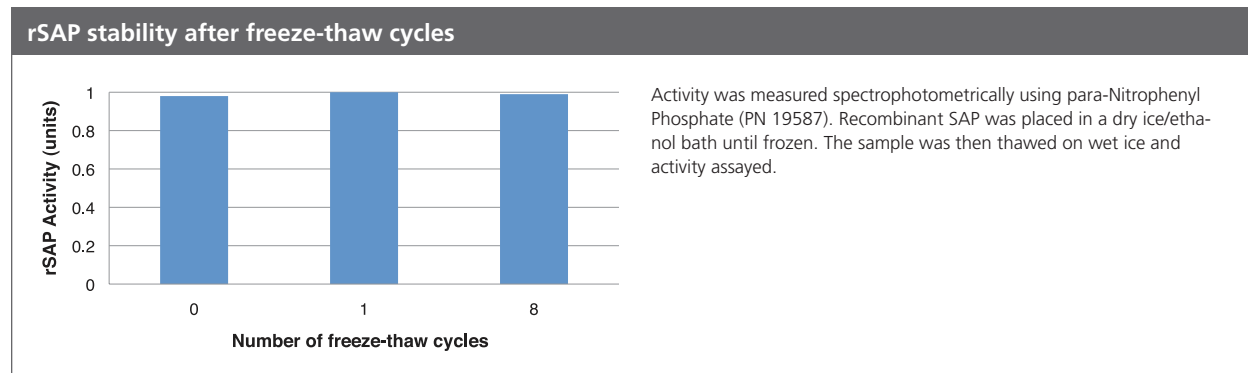
What are the properties of recombinant SAP and native SAP?

See the table and data set below for a comparison of the most relevant attributes.

Property	Recombinant SAP	Native SAP
Molecular weight	Homodimer of \approx 55 kDa monomers	Homodimer of \approx 55 kDa monomers
pH optimum	10.4 in Glycine buffer and 8.0 in Tris-HCl buffer	10.4 in Glycine buffer and 8.0 in Tris-HCl buffer
Reaction temperature	37°C	37°C
Heat-inactivation at 65°C	Half-life \approx 45 seconds and completely inactivated in 5 minutes	Half-life \approx 45 seconds and completely inactivated in 5 minutes
Storage buffer	25 mM Tris-HCl, pH 7.5 1.0 mM MgCl ₂ 50% glycerol	25 mM Tris-HCl, pH 7.6 1.0 mM MgCl ₂ 0.1 mM ZnCl ₂ 50% glycerol
Glycerol content	50% glycerol	50% glycerol
Concentration	1 unit/ μ l	1 unit/ μ l
Source	Recombinant, expressed in yeast	<i>Pandalus borealis</i> (Cold-water shrimp)
Efficiency in:		
Dephosphorylation of 1 μ g cut plasmid (5'-overhang, recessed, and blunt ends)	> 99% after 15 minutes incubation at 37°C using 1 unit	> 99% after 15 minutes incubation at 37°C using 1 unit
Degradation of 2 μ mol nucleotides	> 99% after 15 minutes incubation at 37°C using 2 units	> 99% after 15 minutes incubation at 37°C using 2 units

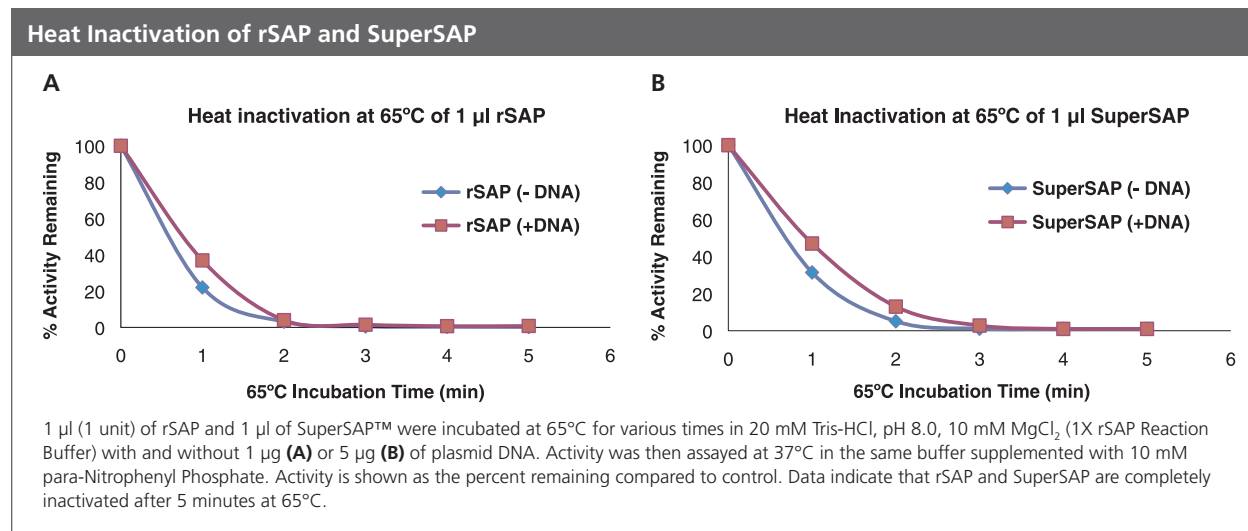
Is rSAP stable after multiple freeze-thaw cycles?

Yes, rSAP retains >99% of its activity after 8 freeze-thaw cycles as shown in the data below.



What is the half-life of rSAP at 65°C in its reaction buffer?

The half-life of rSAP is about 45 seconds at 65°C and is completely inactivated after 5 minutes at 65°C when assayed in its reaction buffer.



Is rSAP active in restriction enzyme buffers?

Yes, rSAP is active when added directly to common restriction enzyme buffers (e.g. NEBuffer 1-4). This was functionally tested by determining the decrease in bacterial colonies after dephosphorylation of linearized vector.

Reaction buffer	Treated/untreated CFU's	% Background reduction
SAP reaction buffer	01/760	100%
NEBuffer #1 restriction buffer	0/760	100%
NEBuffer #2 restriction buffer	2/760	99.7%
NEBuffer #3 restriction buffer	3/760	99.6%
NEBuffer #4 restriction buffer	2/760	99.7%
NEBuffer EcoRI restriction buffer	3/760	99.6%

Pst I digested pUC19 vector was purified using a spin column and resuspended in 10 mM Tris-HCl, pH 8.5. 1 µg of DNA was treated with 1 unit of rSAP in the indicated buffers in 10 µl of pUC19 for 30 minutes at 37°C followed by heat inactivation at 65°C for 15 minutes. 50 ng was self-ligated using the Ligate-IT™ Rapid Ligation Kit (PN 78400) and the equivalent of 2.5 ng was transformed into *E. coli* DH5-α (0.5 ng was plated onto selective medium and bacterial colonies were counted).

Does rSAP efficiently dephosphorylate blunt, overhanging, and recessed 5'-dsDNA ends?

Yes, rSAP does dephosphorylate different dsDNA ends for efficient cloning of all restriction digests as shown in the table below.

5-End state	Treated/untreated CFU's	% Background reduction
5'-overhang (EcoR I)	7/9440	99.9%
5'-recessed (Pst I)	0/6240	100%
Blunt-end (Hinc II)	6/2990	99.8%

Pst I digested pUC19 vector was purified using a spin column and resuspended in 10 mM Tris-HCl, pH 8.5. 1 µg of DNA was treated with 1 unit of rSAP in the indicated buffers in 10 µl of pUC19 for 15 minutes at 37°C followed by heat inactivation at 65°C for 15 minutes. 50 ng was self-ligated using the Ligate-IT™ Rapid Ligation Kit (PN 78400) and the equivalent of 2.5 ng was transformed into *E. coli* DH5-α (0.5 ng was plated onto selective medium and bacterial colonies were counted).

Does vector DNA need to be purified after treatment with rSAP and before ligation?

No, it is not necessary to purify vector DNA after treating with rSAP. However, rSAP must be heat-inactivated by incubating at 65°C for at least 5 minutes prior to adding ATP and insert DNA.

What are the divalent cation requirements for rSAP activity?

Each rSAP monomer possesses three separate metal binding sites: two bind Zn²⁺ ions exclusively and one binds Zn²⁺ or Mg²⁺ ions. The rSAP storage buffer supplies all of the divalent cations that rSAP requires.

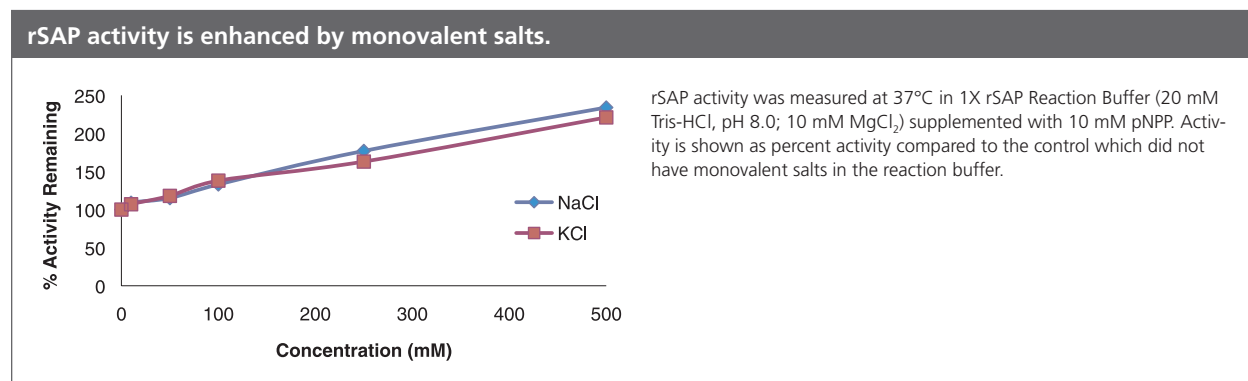
Is rSAP active in reducing agents?

Yes, rSAP activity was measured in 1X rSAP reaction buffer (pH 8.0) with added Dithiothreitol (DTT) or β -mercaptoethanol (β -ME). rSAP retained over 90% of its activity in 1 mM reducing agent and over 60% of its activity in 10 mM.

Reducing Agents	Concentration	rSAP Activity
DTT	1 mM	92%
	5 mM	76%
	10 mM	64%
β -ME	1 mM (0.05%)	98%
	5 mM (0.25%)	77%
	10 mM (0.50%)	62%

Are there salt requirements for rSAP activity?

rSAP is active without monovalent salts, however, the addition of NaCl or KCl will enhance its activity as shown in the figure below.



In what detergents is rSAP active?

rSAP activity was tested in 1X rSAP Reaction Buffer (pH 8.0) with several commonly-used detergents as shown in the table below.

Name	Type	Concentration	rSAP Activity
NP-40	Non-ionic	0.5%	95%
		1%	90%
Tween®-20	Non-ionic	0.5%	91%
		1%	83%
Triton® X-100	Non-ionic	0.5%	84%
		1%	74%
CHAPS	Zwitterionic	0.5%	99%
		1%	93%
SDS	Ionic	0.5%	96%
		1%	91%

Is rSAP active in the presence of Betaine and Dimethyl sulfoxide (DMSO)?

rSAP activity was tested in 1X rSAP Reaction Buffer (pH 8.0) with Betaine and DMSO and is shown in the table below.

Agents	Concentration	rSAP Activity
Betaine	0.5 M	92%
	1 M	77%
DMSO	2%	97%
	3%	92%

What is the optimal pH for rSAP activity?

rSAP is maximally active at pH 10.4 in glycine buffer and at pH 8 in Tris buffers.

Is it possible to dephosphorylate proteins with rSAP?

The literature contains many examples of protein dephosphorylation using either calf^(2,3) or native shrimp^(1,4) alkaline phosphatase. In addition, rSAP activity in the presence of additives, such as detergents and reducing agents, has been demonstrated.

After the PCR reaction, can I add rSAP directly to the reaction to prepare PCR reactions for sequencing?

To prepare PCR reactions for sequencing, add 2 µl of ExoSAP-IT® per 5 µl of PCR reaction. rSAP will only degrade nucleotides. The primers will remain.

If I buy an alkaline phosphatase or exonuclease I from someone other than Affymetrix do I also get a license to the methods of PCR cleanup claimed in these patents?

No, USB ExoSAP-IT and the distributor, GE Healthcare, are the only vendors that provide a license to these patents. Purchase of USB ExoSAP-IT [PN 78200] includes a license to US Patents 5,741,676, 5,756,285, 6,379,940 and 6,387,634 and all foreign equivalents.

References:

1. Wadler, C. and Cronan, J. E. (2007) *Anal. Biochem.*, **368**(1), 17-23.
2. Hirschberg, D., Jagerbrink, T., Samskog, J., Gustafsson, M., Stahlberg, M., Alvelius, G., Husman, B., Carlquist, M., Jornvall, H. and Bergman, T. (2004) *Anal. Chem.* **76**(19), 5864-5871.
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