For life science research only.
Not for use in diagnostic procedures.

rAPid Alkaline Phosphatase

Orthophosphoric-monoester phosphohydrolase (alkaline optimum)

Version: 04

Content Version: November 2021

Cat. No. 04 898 133 001	1,000 U 1 U/µl 1,000 dephosphorylation reactions with a final reaction volume of 20 µl each
Cat. No. 04 898 141 001	5,000 U 1 U/µl 5,000 dephosphorylation reactions with a final reaction volume of 20 µl each

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	rAPid Alkaline Phosphatase, rAPid Alkaline Phosphatase Buffer, 10x conc.	0.5 M Tris-HCl, 1 mM EDTA, pH 8.5 (+20°C).	04 898 133 001	1 vial, 1 ml
			04 898 141 001	
2	rAPid Alkaline Phosphatase, rAPid Alkaline Phosphatase, 1 U/µl	25 mM Tris-HCl, 1 mM $MgCl_2$, 1 mM $ZnCl_2$, 50% glycerol (v/v); pH approximately 7.6 at +4°C.	04 898 133 001	1 vial, 1,000 U
			04 898 141 001	1 vial, 5,000 U

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	rAPid Alkaline Phosphatase Buffer, 10x conc.	Store at −15 to −25°C.
2	rAPid Alkaline Phosphatase, 1 U/µl	

1.3. Application

rAPid Alkaline Phosphatase is used to dephosphorylate 5′ ends of DNA and RNA. Alkaline phosphatase treatment prevents self-ligation of fragments by removing the 5′-phosphoryl termini required by ligases; this feature is of major importance in cloning strategies to decrease vector background.
Use rAPid Alkaline Phosphatase in numerous applications, including:

- Removal of 5′-phosphoryl groups from nucleic acids.
- Preparation of templates for 5′-end labeling.
- Clean-up of PCR products by removal of dNTPs.
- Dephosphorylation of protein.

1.4. Preparation Time

Assay Time

The assay time depends on the nature of the DNA ends to be dephosphorylated.

Dephosphorylation

- Up to 1 µg DNA with blunt or sticky 5′-protruding ends in 10 minutes at +37°C.
- Up to 1 µg DNA with sticky 5′-recessive ends in 30 minutes at +37°C.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Background information

DNA ligases join linear DNA fragments together via covalent bonds. DNA ligation involves creating a phosphodiester bond between the 3′ hydroxyl group of one nucleotide and the 5′ phosphate of another. Ligation of DNA fragments is an essential step in many molecular biology techniques, including gene cloning and messenger RNA (mRNA) fingerprinting. For efficient ligation, DNA strands must be prevented from self-ligating (self-circularization and concatenation) by dephosphorylation of DNA ends. Alkaline phosphatase removes the 5′-phosphoryl termini required by ligases, preventing self-ligation of DNA and decreasing background.

No additional purification steps are required after restriction and dephosphorylation. The dephosphorylated DNA can directly be used in a ligation reaction.

2.2. Protocols

Standard dephosphorylation

1. Thaw all necessary components.
   – Briefly centrifuge all reagents before starting.

2. Add the following reagents to a reaction vial:

Reagent	Volume [µl]	Final concentration
Vector DNA	variable	up to 1 µg
rAPid Alkaline Phosphatase Buffer, 10x conc.	2	1x
rAPid Alkaline Phosphatase	1	1 U
Double-distilled water	add to a final volume of 20 µl	–
Total Volume	**20**	

– Mix thoroughly and centrifuge briefly.

3. Incubate DNA with blunt or sticky 5′-protruding ends for 10 minutes at +37°C.
   – Incubate DNA with sticky 5′-recessive ends for 30 minutes at +37°C.

4. Inactivate the rAPid Alkaline Phosphatase for 2 minutes at +75°C.

5. Either use the dephosphorylation reaction mixture directly in the following ligation reaction or store it at −15 to −25°C until further use.

2.3. Parameters

EC-Number

EC 3.1.3.1

Inactivation

rAPid Alkaline Phosphatase is inactivated by incubation at +75°C for two minutes.

Molecular Weight

56 kD (SDS-PAGE), monomer

Homodimer; Zn^{2+} is essential for activity.

Specific Activity

1 U/µg

Specificity

Alkaline Phosphatase catalyzes the hydrolysis of numerous phosphate esters, such as esters of primary and secondary alcohols, saccharides, cyclic alcohols, phenols, and amines.

- Phosphodiesters do not react.
- The enzyme hydrolyzes inorganic pyrophosphate.
- The kinetic properties of the enzyme depend on many factors, such as purity of enzyme, concentration of enzyme in the assay, buffer, pH etc.

Temperature Optimum

+37°C

Unit Definition

One unit of rAPid Alkaline Phosphatase is the enzyme activity which hydrolyzes 1 µmol of 4-nitrophenyl phosphatase in one minute at +37°C under assay conditions.

Volume Activity

1 U/µl

3. Additional Information on this Product

3.1. Test Principle

rAPid Alkaline Phosphatase catalyzes the dephosphorylation of 5′ phosphates from DNA and RNA, nucleotides, and proteins.

- Unlike calf intestinal phosphatase, rAPid Alkaline Phosphatase is rapidly, completely, and irreversibly inactivated by heat treatment for two minutes at +75°C. It is therefore an excellent alternative to Shrimp Alkaline Phosphatase.
- In addition, the enzyme is active in restriction enzyme buffers; therefore, restriction enzyme digestion, dephosphorylation, enzyme inactivation, ligation, or 5′-end labeling can be performed without purification steps.

Preparation

rAPid Alkaline Phosphatase is isolated from bovine intestine and supplied as a recombinant enzyme expressed in the yeast, *Pichia Pastoris*. The recombinant form ensures consistency and safety. There are no animal-derived additives.

3.2. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Trademarks

All product names and trademarks are the property of their respective owners.

4.4. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products and select the corresponding product catalog.

4.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed

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